l States
tment of
ulture

al Grain
tion
ervice

February 21, 1992

Aflatoxin Handbook

United States Department of Agriculture
Federal Grain Inspection Service

Program Handbook

February 21, 1992

Foreword

The Federal Grain Inspection Service provides aflatoxin testing services under the authority of the United States Grain Standards Act, as amended (USGSA), and under the authority of the Agricultural Marketing Act of 1946, as amended (AMA). The Aflatoxin Handbook provides policies and procedures for these aflatoxin services.

This handbook is revised to reflect the mandatory export corn aflatoxin testing provision and the testing of grains for aflatoxin under the authority of the USGSA as noted in the January 22, 1992, Federal Register final rule (57 FR 2438).

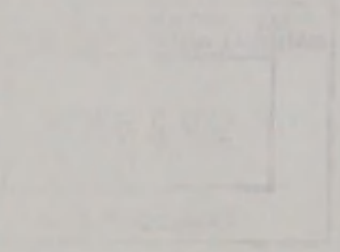
Replace the Aflatoxin Handbook, dated 1/1/90, and subsequent changes, dated 2/14/90, 8/7/90, and 5/16/91, with this new handbook. The new handbook also supersedes Program Bulletin 91.6, Aflatoxin Testing Service, dated 7/12/91.

John W. Marshall

Director
Field Management Division

Distribution: A, C, E, U

Originating Office: SPB, FM



U.S. DEPARTMENT OF AGRICULTURE
Grain Inspection, Packers and Stockyards Administration
Federal Grain Inspection Service
P.O. Box 96454
Washington, D.C. 20090-6454

TABLE OF CONTENTS

U.S. DEPARTMENT OF AGRICULTURE
Federal Grain Inspection Service
P.O. Box 96454
Washington, D.C. 20090-6454

CHAPTER 1

GENERAL INFORMATION

1.1
SCOPE

This handbook establishes procedures for determining and certificating aflatoxin in all grains and commodities assigned to the Federal Grain Inspection Service (FGIS) and monitoring the performance of the FGIS aflatoxin testing program.

Official aflatoxin testing of all grains and grain products is provided, throughout the United States, at various FGIS field offices, delegated and designated agencies, State cooperators, and the FGIS Commodity Testing Laboratory (CTL) in Beltsville, Maryland.

Official personnel perform all official aflatoxin testing services in accordance with procedures prescribed in this handbook. Only authorized or licensed employees provide official aflatoxin testing services using approved aflatoxin testing methods.

A. Aflatoxin Testing Services Under the United States Grain Standards Act (USGSA).

The 1990 Farm Bill (Food, Agriculture, Conservation, and Trade Act of 1990, P.L. 101-624) amended section 5 of the USGSA to "... require that all corn exported from the United States be tested to ascertain whether it exceeds acceptable level of aflatoxin contamination, unless the contract for export between the buyer and seller stipulates that aflatoxin testing shall not be conducted."

Aflatoxin testing services are available, upon request, for all grains under the authority of the USGSA. Mandatory aflatoxin testing is required only for corn exported from the United States. FGIS tests and certifies aflatoxin as low as 5 parts per billion (ppb) using the Aflatest method. Testing and certification of export shipments at lower levels is permitted on a case-by-case basis.

In some instances, the demand for aflatoxin testing services may not warrant the expense of establishing and equipping an aflatoxin laboratory by delegated and designated agencies. If this occurs, the agency manager may locate a mutually agreeable agency to provide service when necessary. If an agency cannot provide for adequate service, FGIS will determine, on a case-by-case basis, if FGIS will provide the service.

B. Aflatoxin Testing Services Under the Agricultural Marketing Act (AMA).

Aflatoxin testing service is available, upon request, for agricultural commodities and products assigned under the authority of the AMA.

1.2 BACKGROUND

Aflatoxin is a naturally occurring mycotoxin produced by two types of mold known as Aspergillus flavus and Aspergillus parasiticus. A. flavus is very common and widespread in nature and is more likely to occur when certain grains are grown under stressful conditions such as drought. It occurs in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration. It invades all types of organic substrates whenever and wherever the conditions are favorable for its growth. Favorable conditions include high moisture content and high temperature. At least 13 different types of aflatoxin are produced in nature. Aflatoxin B1 is considered by many as the most toxic.

1.3 LABORATORY SAFETY

FGIS personnel must abide by all safety and health rules referenced in this handbook.

Interested persons are prohibited from entering the aflatoxin testing laboratory area during testing unless accompanied by official personnel and must observe the health and safety rules while in the laboratory.

During onsite supervision at agency locations, FGIS employees must assess their personal safety requirements. If personal safety is questionable, FGIS employees must determine if personal protective equipment can be used to correct the safety deficiency at the laboratory. If FGIS employees cannot utilize personal protective equipment to provide for a safe work environment, then onsite supervision must occur when aflatoxin testing is not being performed and the laboratory is considered safe.

1.4 DISCLAIMER CLAUSE

The mention of firm names or trade products does not imply that the U.S. Department of Agriculture endorses or recommends them over other firms or similar products not mentioned.

1.5 RESPONSIBILITIES

The general responsibilities for the aflatoxin testing program are as follows:

A. Responsibilities of the Quality Control and Testing Branch Chief.

1. Provide analytical support to the Board of Appeals and Review for Board appeal inspections under USGSA.

2. Monitor the accuracy of the aflatoxin testing services provided by the national inspection system.

3. Review aflatoxin testing procedures at FGIS field offices, delegated and designated agencies, and State Cooperators, when requested.

4. Provide technical support and training to official inspection personnel in matters relating to aflatoxin testing.

5. When needed, initiate collaborative and/or special studies and report the findings.

6. Recommend follow-up action when problems are detected.

B. Responsibilities of the Board of Appeals and Review Chairperson.

1. Provide Board appeal inspection services for aflatoxin tests conducted under the authority of the USGSA. (QCTB provides analytical support.)

2. Issue certificates and assess fees for Board appeal inspection services.

C. Responsibilities of the Commodity Testing Laboratory (CTL) Manager.

1. Provide aflatoxin testing services for all USGSA and AMA products except when a field laboratory has the ability to provide such service.

2. Prepare and issue certificates, when necessary.

3. Monitor the accuracy of the thin-layer chromatography (TLC) method at the CTL and field laboratories that perform TLC testing.

D. Responsibilities of FGIS Field Office Managers.

1. General.

a. Review safety procedures at FGIS aflatoxin testing laboratories within the circuit and, when necessary, make recommendations.

b. Maintain the aflatoxin testing program and serve as the primary contact within the circuit.

c. Select and forward monitoring samples to QCTB and assist QCTB in monitoring agencies within the circuit.

d. Review aflatoxin testing procedures at specified service points within the circuit.

e. Provide technical support and training to official inspection personnel and cooperators.

f. Assist QCTB in conducting collaborative and/or special studies.

g. Inform QCTB and Field Management Division of problems detected in the circuit and initiate follow-up action.

2. Grain (USGSA).

a. Provide or arrange to provide for original inspection and reinspection services:

(1) in areas not assigned to delegated or designated agencies, or

(2) on a case-by-case basis and with the approval of Field Management Division, in areas where an official agency is unable to provide service.

b. Provide or arrange to provide for all appeal inspection services within the circuit.

c. Forward file samples to the Board of Appeals and Review for Board appeal inspection services.

3. Commodities (AMA).

a. Provide or arrange to provide original and retest inspection services for certain commodities based on testing capabilities.

b. Provide or arrange to provide appeal inspection services.

E. Responsibilities of Delegated or Designated Agency Managers.

1. Consult with local authorities to ensure that all aflatoxin testing laboratories in the assigned geographic area meet local safety, health, and environmental requirements.

2. Provide original and reinspection aflatoxin services within assigned geographic area.

3. Forward file samples for appeal inspection services to the field office or as directed by the field office manager.

4. Select and forward monitoring samples to QCTB or as directed by the field office manager.

5. Routinely review aflatoxin testing procedures at specified service points within the assigned geographic area.

6. Permit only official personnel that are trained and licensed for aflatoxin testing to perform such activities.

7. Provide technical support and training to licensed inspection personnel within the assigned geographic area.

8. Assist QCTB and FGIS field office in conducting collaborative and/or special studies.

9. Inform the field office manager of problems detected within the assigned geographic area and initiate corrective and follow-up actions.

F. Responsibilities of State Cooperators.

1. Consult with local authorities to ensure that all aflatoxin testing laboratories in the assigned geographic area meet local safety, health, and environmental requirements.

2. Provide original and retest aflatoxin testing services within the State.

3. Forward file samples for appeal inspection services to an equipped FGIS field office or to CTL as directed by the field office manager.

4. Select and forward monitoring samples to QCTB or CTL as directed by the respective field office manager.

5. Routinely review aflatoxin testing procedures at specified service points within the State.

6. Permit only official personnel that are trained and licensed for aflatoxin testing to perform such activities.

7. Provide technical support and training to licensed inspection personnel within the State.

8. Assist QCTB and FGIS field office in conducting collaborative and/or special studies.

9. Inform the FGIS field office manager of problems detected within the State and initiate corrective and follow-up actions.

1.6 REQUEST FOR SERVICE

Individuals wanting grains or commodities tested for aflatoxin should contact the nearest FGIS field office, delegated or designated agency, or authorized State Cooperator to arrange for sampling and testing. Individuals may also obtain aflatoxin testing services by submitting a sample to any FGIS field office, delegated or designated agency, or authorized State Cooperator.

Delegated and designated agencies provide aflatoxin testing services for grains under the USGSA only. State Cooperators provide aflatoxin testing services for AMA products, such as corn meal and corn/soy blend etc. If a field office, agency, or State Cooperator are unable to pro de aflatoxin testing services for a particular product, a sample will be mailed to the Kansas City field office or to CTL for analysis.

1.7 AVAILABLE INSPECTION SERVICES

Applicants have various aflatoxin testing service options based on the kind of product tested.

A. Grain (USGSA). The following kinds of aflatoxin testing services are provided under the authority of the USGSA:

1. Official Sample-Lot Inspection Service. This service consists of official personnel sampling and testing an identified lot of grain.

2. Warehouseman's Sample-Lot Inspection Service. This service consists of a licensed warehouseman sampler sampling an identified lot of grain using an approved diverter-type mechanical sampler and sending the sample to official personnel for testing.

3. Submitted Sample Inspection Service. This service consists of official personnel testing a grain sample submitted by the applicant.

B. Commodities (AMA). The following kinds of aflatoxin testing services are provided under the authority of the AMA:

1. Quality Inspection Service. This service consists of official personnel sampling and testing an identified commodity lot.

2. Submitted Sample Inspection Service. This service consists of official personnel testing a sample submitted by the applicant.

1.8 TESTING METHOD-OLOGIES

FGIS has approved the following testing methods and test kits for aflatoxin determinations in corn. As noted, some test methods are also approved for other grains and commodities.

METHOD AND TEST KITS	APPROVED FOR QUALITATIVE	APPROVED FOR QUANTITATIVE
1. Thin-layer Chromatography (TLC).	X	X
2. Afla-20-Cup (International Diagnostics, Inc.	X	
3. Aflatest (VICAM).	X	X
4. CITE Probe (IDEXX Corporation).	X	
5. EZ-Screen (Environmental Logistics, Inc.).	X	
6. Agri-Screen (Neogen Corporation).	X	
* 7. Veratox AST (Neogen Corporation).	X	X
8. Oxoid (Oxoid, U.S.A., Inc.).	X	
9. Sam-A (Rialdon Diagnostics).	X	
10. Holaday Velasco Minicolumn (MC)	X	
11. Modified Minicolumn (MMC)	X	

Note: The TLC method is also approved for all other grains, rice, and processed products. The Aflatest and the Veratox AST methods are also approved for sorghum, soybeans, wheat, rice, popcorn, corn meal, corn germ meal, corn gluten meal, and corn/soy blend.

For an updated listing of approved aflatoxin testing methods and test kits, contact the nearest FGIS field office.

In addition, the ultraviolet light (blacklight) may be used to test for the presence of the fungus A. flavus as a presumptive test. The blacklight is not recognized by FGIS as a qualitative or quantitative test for aflatoxin and it does not fulfill the USGSA requirement for testing export corn for the presence of aflatoxin. Agencies may offer a blacklight testing service to their customers on an unofficial basis, providing the service is limited to domestic movements and the customer is aware that this testing method does not fulfill the USGSA requirement for testing export lots of corn for the presence of aflatoxin.

(Revised 6-30-95)

1.9
REVIEW
INSPECTIONS

The levels and methods of testing services available to an applicant vary based on the testing authority and the reason for the review. Refer to charts 1 and 2 for review inspection procedures under USGSA for single-lots and material portions and refer to chart 3 for review inspection procedures under AMA.

A. Grain (USGSA). Review inspection services for aflatoxin are provided on either a new sample or the file sample in accordance with the regulations. Board appeal inspection services are limited to the analysis of file samples. Only one field review (reinspection or appeal inspection) is permitted for shiplot, unit train, or lash barge material portions when sublots are tested. All review inspection results replace previous results.

Sections 800.125 and 800.135 of the USGSA regulations permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factors, and official criteria may be handled separately even though both sets of results are reported on the same certificate. When official grade or official factors and official criteria are reported on the same certificate, the review inspection certificate shall show a statement indicating that the review results are for official grade, official factors, or official criteria and that all other results are those of the original, reinspection, appeal inspection, or Board appeal inspection results, whichever is applicable.

If a review inspection of the same level or lower level is requested on the other results, the original and all copies of the effected certificate(s) must be obtained by official personnel. Mark the effected certificate(s) "Void" and issue a new certificate(s). If the original and all copies of the effected certificates are not obtained, the request is denied.

1. Reinspection Service. The laboratory providing original testing services also provides reinspection services. The testing laboratory may use any approved test method available for the reinspection unless the original test was quantitative. Then, only a quantitative reinspection is available.

2. Appeal Inspection Service. Only quantitative test methods are available for aflatoxin appeal tests.

a. FGIS field offices provide appeal aflatoxin testing services if adequate test equipment is available. Samples are sent to the Kansas City field office when field offices are not equipped with quantitative testing equipment.

b. Applicants interested in an appeal aflatoxin test based on the TLC method may request that the sample be sent to an FGIS TLC-equipped field office or CTL.

When sending samples to the Kansas City field office or CTL, write the words "AFLATOXIN APPEAL" in the "Remarks" section of the grain sample ticket and on the back of the mailing tag.

3. Board Appeal Inspection Service. Only quantitative test methods are available for aflatoxin Board appeal tests and the service is limited to the file sample.

When sending samples to the Board of Appeals and Review, write the words "AFLATOXIN BOARD APPEAL" in the "Remarks" section of the grain sample ticket and on the back of the mailing tag.

CHART NO. 1 - USGSA REVIEW INSPECTION : SINGLE-LOT

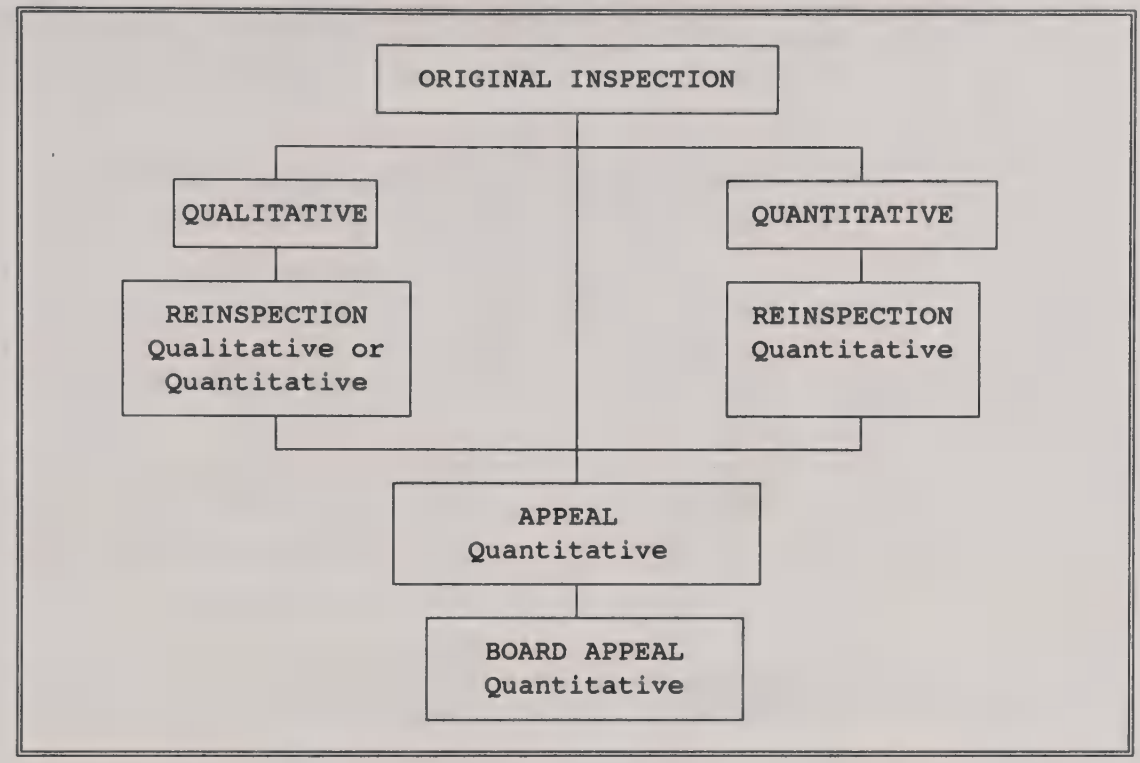
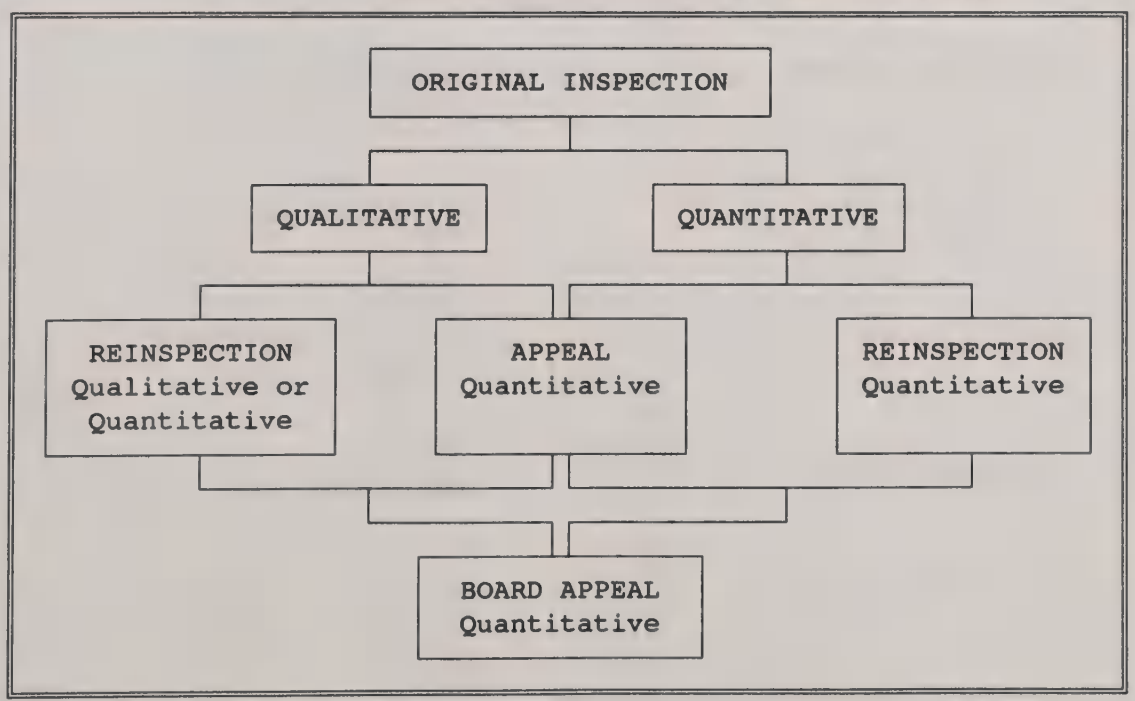


CHART NO. 2 - USGSA REVIEW INSPECTION : MATERIAL PORTIONS
(Shiplots, Unit Trains, and Lash Barges)



B. Commodities (AMA). Review inspection services for aflatoxin are limited to the analysis of file samples. Review inspection results replace previous results.

1. Retest Inspection Service. The laboratory providing original testing services also provides retest services. The testing laboratory may use any approved test method available for the retest unless the original test was quantitative. Then, only a quantitative retest is available.

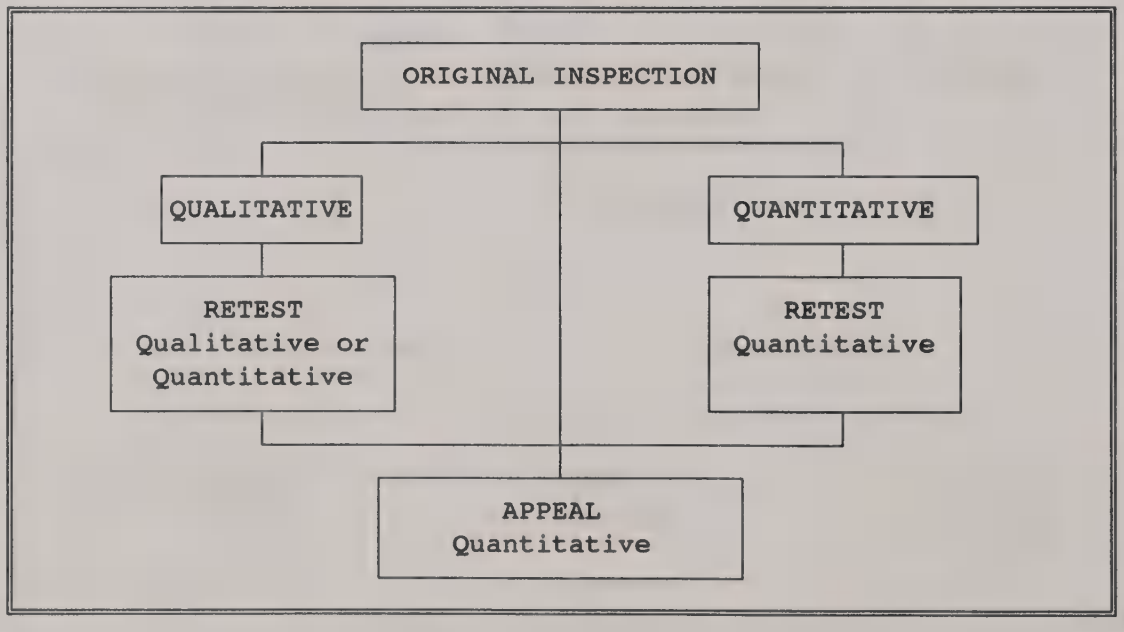
2. Appeal Inspection Service. Only quantitative test methods are available for aflatoxin appeal tests.

a. FGIS field offices provide appeal aflatoxin testing services if adequate test equipment is available. File samples are sent to the Kansas City field office or CTL when field offices are not equipped with quantitative testing equipment.

b. Applicants interested in an appeal aflatoxin test based on the TLC method may request that the official file sample be sent to an FGIS TLC-equipped field office or CTL.

c. CTL provides appeal aflatoxin testing services for processed products normally tested at CTL and for products which cannot be handled at field locations.

CHART NO. 3 - AMA REVIEW INSPECTION



1.10
SAMPLING
AND TESTING
UNIT TRAINS

A. Requesting Service. Applicants must specify the following when requesting aflatoxin testing services for unit trains:

1. Sampling Basis. Samples are obtained and tested on either an individual carrier basis or a sublot basis (up to five carriers per sublot). When articulated railcars are used, each car is tested as a sublot. Applicants may request aflatoxin testing on a sublot basis while requesting inspection for grade on an individual carrier basis. If this occurs, factor only certificates are issued for aflatoxin and individual grade certificates are issued for each carrier. Export corn shipments are automatically tested on a sublot basis unless the applicant indicates official testing is not required or an individual carrier basis is needed.

2. Testing Basis. Indicate if qualitative or quantitative testing is needed.

3. Aflatoxin Specification. Indicate the maximum acceptable aflatoxin limit if different than 20 ppb.

4. Certificate Issuance. Indicate if a certificate representing each sample tested or the entire unit train is needed. When grade and aflatoxin are provided on the same sampling basis, indicate if grade and aflatoxin are reported on one certificate or separate certificates.

B. Sampling and Testing Procedures.

1. Individual Carrier Basis. Obtain a 3-pound sample from each carrier and test each sample for aflatoxin.

2. Sublot Basis.

a. Combine carriers to form sublots in the order of loading or unloading. Applicants may not request a combination of carriers which are not consecutively loaded or unloaded.

b. Obtain a representative sample from each carrier to make a 10-pound sublot sample. Proportionately sample carriers to form the sublot sample.

c. Analyze the 10-pound sublot sample to determine if it meets contract specification. A material portion occurs if the sublot result exceeds 20 ppb or a limit specified by the applicant if different than 20 ppb.

d. The applicant may request review procedures when a material portion occurs according to the procedures in section 1.9. Review inspection results replace previous results when determining if a material portion exists.

C. Unit Train Certification.

1. Individual Test Results. Unless otherwise specified by the applicant, certificate each test result on a separate certificate. A certificate may represent a single carrier or a sublot from up to five carriers. The sublot certificate lists the identities of the carriers comprising the sublot.

2. Combined Test Results. At the request of an applicant, individual test results that do not exceed the maximum acceptable aflatoxin limit, e.g., 20 ppb, may be combined in the case of qualitative tests or averaged in the case of quantitative tests and reported on a single certificate. Results exceeding the maximum aflatoxin limit are certificated separately for each test result.

For example, an applicant requests one quantitative certificate showing the identification of all railcars in a 50-car unit train. Official personnel would:

a. Sample all 50 railcars and make 10 sublot samples by combining the individual samples from each 5 railcars in sequential order.

b. Test the 10 sublot samples for aflatoxin using a quantitative test.

c. Record the 10 sublot results on a work log to the nearest ppb.

d. Average all results from the sublots that do not exceed the maximum acceptable aflatoxin limit.

e. Issue a certificate for the averaged results and issue a separate certificate for any sublot exceeding the maximum aflatoxin limit.

For additional information on the certification of results, see chapter 15.

1.11 TESTING EXPORT SHIPLOTS

The USGSA requires aflatoxin testing for all corn exported from the United States. Export corn is not officially tested for aflatoxin if the contract stipulates testing by an entity other than FGIS or if official testing is not required. Corn contracts which are silent with respect to aflatoxin are not exempt from mandatory aflatoxin testing.

A. Requesting Service. Applicants must specify the following when requesting aflatoxin testing services for export shiplots:

1. Sampling Basis. Corn and sorghum are always tested for aflatoxin on a sublot basis. Other grains and commodities are tested for aflatoxin on a composite sample basis. Applicants may request composite sample analysis in addition to the sublot test when corn and sorghum are exported. Applicants may also request sublot testing instead of composite sample analysis for other grains and commodities routinely tested on a composite basis.

2. Testing Basis. Indicate if qualitative or quantitative testing is needed.

3. Aflatoxin Specification. Indicate the maximum acceptable aflatoxin limit if different than 20 ppb.

4. Certificate Issuance. Indicate if grade and aflatoxin are reported on one certificate or separate certificates.

B. Sampling and Testing Procedures.

1. Sublot Basis.

a. Obtain and test a 10-pound sample from each sublot.

b. Record each sublot result on the inspection log or similar document. If requested by the applicant, quantitative results used as a screening process are reported on the inspection log as screening results (exceeding or less than or equal to the maximum level) provided that laboratory records are maintained in actual ppb. This is necessary for proper quality control and monitoring.

c. A material portion occurs if the sublot result exceeds 20 ppb or a limit specified by the applicant if different than 20 ppb.

d. The applicant may request review procedures when a material portion occurs according to the procedures in section 1.9. Review inspection results replace previous results when determining if a material portion exists.

e. If a material portion designation due to aflatoxin is not removed by the review inspection process, the applicant may:

(1) leave the material portion on the vessel and receive a separate certificate,

(2) return the grain/commodity from a shipping bin to the elevator,

(3) discharge the material portion along with additional grain/commodity in common stowage equivalent to half the material portion quantity.

2. Composite Basis. Obtain a sample during loading in sufficient quantity to make a 10-pound composite sample which represents the export lot. Test the composite sample for aflatoxin upon completion of loading.

C. Shiplot Certification. Certificate shiplots based on the following guidelines and chapter 15 of this handbook.

1. Qualitative Certification. When sublot samples are tested using a qualitative method, certificate the shiplot as equal to or less than the maximum limit, e.g., 20 ppb. Include on the same certificate the composite sample results (qualitative or quantitative) if a composite sample was also tested. If some sublots were reviewed using a quantitative method, continue to certificate the shiplot as equal to or less than the maximum limit.

2. Quantitative Certification. When sublot samples are tested using a quantitative method, certificate the shiplot based on the mathematical or weighted average of the accepted sublot results (See book III of the Grain Inspection Handbook, Section 2.8, "Determining Mathematical or Weighted Average"). In addition, include on the same certificate the composite sample results (qualitative or quantitative) if requested. Separately certificate material portions.

For additional information on the certification of results, see chapter 15.

U.S. DEPARTMENT OF AGRICULTURE
Federal Grain Inspection Service
P.O. Box 96454
Washington, D.C. 20090-6454

CHAPTER 2

LABORATORY PRACTICES AND RESPONSIBILITIES

2.1 GENERAL FGIS LABORATORY PRACTICES

When working in a laboratory, FGIS employees must comply with good laboratory practices to ensure a safe and efficient work environment. To accomplish this, include the following as part of an overall FGIS laboratory "Standard Operating Procedure" (SOP) (see section 2.4). Maintain the SOP, this handbook, and current Material Safety Data Sheets (MSDS) at each laboratory.

A. Label all bottles and containers according to the Hazard Communication Program. In addition, when preparing mixtures of solutions, securely apply a label with the name of the solution, the preparation date, and the preparer's initials written in permanent ink.

B. Do not smoke, eat, drink, or chew gum or tobacco in the laboratory.

C. Wash hands immediately before and after eating, drinking, and smoking outside of the laboratory area.

D. Wear a disposable, fire-retardant laboratory coat and disposable impermeable gloves when working.

E. Decontaminate spills of aflatoxin solution, labware, disposable materials used for analysis, and the work area as prescribed in section 2.3.

F. Wear a disposable mask, (3M Model 8710 or Moldex Model 2200) and hair protection, (Lab Safety Supply EB 1357 or 1356), when exposed to airborne grain dust.

G. Do not wear contact lenses in the laboratory.

H. Wear safety glasses or splash goggles when in the lab. (Also applies to visitors in the lab.)

I. Do not store food or drink in the laboratory refrigerator. Store only the test kits and other items requiring refrigeration.

J. Do not wear protective clothing outside the laboratory unless waste chemicals are being removed to outside storage facilities or extra chemicals are being carried into the laboratory from an outside storage cabinet.

K. Do not store masks and hair protectors in the grinding area where they might become contaminated by the dust particles.

L. Do not use oven for heating food.

2.2 CHEMICALS AND SOLVENTS

A. Store chemicals and equipment outside the hood. Except, if ether is being used, store cans that have had the seal broken in the hood with the hood kept in operation.

B. Store chemicals in places where they will not clutter bench tops or obstruct movements. Do not store solutions at a height exceeding 42 inches above floor level.

C. Prepare all solutions and perform analyses in a working fume hood.

D. Limit the total quantity of waste chemicals in the laboratory to 1 liquid gallon.

E. Maintain a current MSDS for each chemical at the laboratory. If each supply of chemicals received does not have a MSDS enclosed, contact the company and request one immediately.

F. Limit the total amount of flammable solvent in the laboratory to 2 gallons.

G. Store flammable solvents in an approved solvent storage cabinet.

2.3 CLEANLINESS AND DECONTAMINATION PROCEDURES

Perform the following procedures only while wearing disposable impermeable gloves and chemical splash goggles. If hands become contaminated, wash immediately with undiluted bleach followed by soap and water.

A. Spillage. Clean areas and materials contaminated by any aflatoxin solution spills with bleach. The affected area should be completely covered with 5-6 percent sodium hypochlorite (household bleach) dispensed from a plastic wash bottle or spray bottle. Apply 10 parts of bleach to 1 part of spilled material and leave for at least 5 minutes. Wipe up the bleach using an absorbent cloth or paper towels. Place cleaning materials in a plastic waste bag, close tightly, and discard in a dumpster or landfill disposal site.

B. Labware. Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g. 100 mL of bleach to 1000 mL of water). Completely submerge the used glassware, funnels, beakers, etc. and soak for at least 5 minutes. Wash the items and thoroughly rinse with clean water before reusing.

C. Disposable Materials. Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag. Discard in a dumpster or landfill disposal site.

D. Work Area. Decontaminate the work area and all surfaces of the room where corn dust is likely to have settled by wiping the walls and work surfaces with a cloth or paper towels soaked in a bleach solution (1 part bleach to 10 parts water) at the end of each shift.

E. Excess Sample Extract. The sample extract left over after completion of the test procedure is to be decontaminated prior to disposal to the waste drum. Once the analysis has been completed, using a plastic wash bottle, add bleach equal to one half of the volume remaining in the test tube of sample extract and dispose in an approved waste container. Empty contents of waste container in outside waste disposal drum daily. (See chapter 14).

2.4 FIELD OFFICE MANAGER RESPONSIBILITY

A. Supplement this handbook with a SOP for each testing laboratory. The SOP should be tailored to accommodate the individual workload and environment for each location.

B. Develop a Hazard Communication Program for personnel that perform tests involving hazardous materials and ensure that all personnel complete the program.

C. Contact an Environmental Protection Agency (EPA)-approved or EPA-certified waste disposal company and make arrangements for removal of chemical wastes or provide

other suitable waste disposal procedures consistent with existing laws that do not create a hazard to the community.

D. Provide impermeable metal containers meeting Underwriters Laboratory approval for Class I liquids that can be tightly sealed and which are labeled "Flammable" or "Biohazardous Material" or both, as applicable, for storing waste methanol, toluene, acetone, chloroform, and solutions for removal.

E. Provide plastic disposal bags for disposal of decontaminated material such as filter paper, laboratory coats, disposable pipette tips, gloves, etc.

F. Provide containers and labels for disposal of excess grain. Labels are to state "FOR LABORATORY USE ONLY - NOT FOR USE AS FOOD OR FEEDSTUFF," and are to be placed on containers prior to disposal.

G. Provide signs for the laboratory door as follows:

1. "Biohazardous Material Present."

2. "No Smoking, Eating, or Drinking."

3. "Flammable Material Present."

4. "Wear Safety Protection."

5. "Admittance of Authorized Personnel Only."

H. Provide signs for the refrigerator, if present, as follows:

1. "Biohazardous Material Present."

2. "No Food or Drink to be Stored in This Refrigerator."

I. Provide adequate training for laboratory employees prior to performance of laboratory functions to include:

1. Information conveying operations and conditions which can result in exposure to aflatoxin.

2. Contents and availability of Material Safety Data Sheets for relevant chemical agents.

3. Precautions to take when working with aflatoxin contaminated products, including personal hygiene, personal protection equipment, and methods of decontamination.

4. Purpose, proper care, and limitations of dust masks and other protective equipment.

5. Engineering and work practice controls including cleaning methods.

6. Review of the SOP at the laboratory.

7. Proper handling and disposal of waste.

J. Maintain the following safety and health records:

1. Records of any employee injury or illness involving over-exposure to chemicals (29 CFR 1904; 29 CFR 1960.66 through .77b).

2. List of employees trained and assigned to perform aflatoxin tests.

3. Copies of any safety and health studies pertaining to the laboratory.

CHAPTER 3

FGIS LABORATORY DESIGN

4. Separate the space from central heating, ventilation, and air-conditioning using automatic-closing fire dampers in the heating, ventilation, and air-conditioning ducts near the fire barrier, or provide a separate heating, ventilation, and air-conditioning system for the laboratory.

B. Size. Dedicate the space strictly for laboratory (chemical) work. Supply adequate space for chemical analysis (minimum of 100 square feet) and a separate area for sample preparation and grinding purposes. Samples must be ground in space separate from the analytical space.

C. Electrical System. Provide the laboratory space with electrical power and lighting meeting the standards of the National Electrical Code. Wiring suitable for a Class I location is not required. A three-wire system consisting of an energized wire, a neutral wire, and a grounding conductor is satisfactory.

Install overhead lighting fixtures through ceilings that serve as fire barriers. Fixtures suspended below such ceilings are acceptable.

D. Exhaust System. The exhaust system must remove methanol vapors from the work area. Normal air conditioning and heating may provide adequate ventilation when performing testing procedures in a building devoted exclusively for laboratory space. The local Collateral Duty Safety and Health Officer and the Safety and Health Office in Washington, DC, will assist in assessing on a case-by-case basis whether added ventilation, such as a fumehood, is needed. If needed, situate the laboratory space so that hoods, to be supplied by FGIS, are vented to the exterior of the building. Fumehood ventilation will require a 6- or 8-inch diameter opening either vertically through the ceiling and roof or horizontally through an exterior wall. In some cases, a portable hood may be sufficient.

E. Plumbing. Provide the laboratory space with a basin having hot and cold potable water and a sewer connection.

For further information about these requirements, contact the FGIS Safety and Health Staff.

U.S. DEPARTMENT OF AGRICULTURE
Federal Grain Inspection Service
P.O. Box 96454
Washington, D.C. 20090-6454

CHAPTER 4

SAMPLING PROCEDURES AND PROCESSING SAMPLES

4.1 GENERAL

The manner in which samples are obtained and processed is an important consideration when testing for aflatoxin. To ensure that the test results accurately reflect the aflatoxin concentration present in a lot, samples must be representative of the lot and of sufficient size to compensate for the uneven distribution of the contaminant.

4.2 SAMPLING

Obtain samples according to the instruction in the Grain Inspection Handbook, Book I, "Grain Sampling."

The minimum sample sizes based on the type of lot are as follows:

Lot Tupe	Minimum Sample Size
Trucks	2 pounds (approx. 908 grams)
Railcars	3 pounds (approx. 1,362 grams)
Barges/Sublots	10 pounds (approx. 4,540 grams)

A 10-pound sample size is also recommended, but not required, for submitted samples.

CTL will take the portion for aflatoxin testing from the processed products samples received for other quality tests.

4.3 WORK RECORDS

Each testing laboratory must maintain work records for each test that include the name of the applicant, date of service, sample or carrier identification, test results, initials of official personnel performing the test, and any other information deemed necessary to properly certificate the test results and bill the applicant. As practical, use existing forms, such as FGIS-992, "Services Performed Report;" FGIS-920, "Grain Sample Ticket;" or FGIS-921, "Inspection Log," to record laboratory results.

Any sample sent to the Kansas City field office, the Board of Appeals and Review, CTL, or QCTB for aflatoxin testing or monitoring must include the necessary information to facilitate sample processing and testing.

See Chapter 16, "Monitoring," for instructions on sending monitoring samples.

4.4 SAMPLE PREPARATION

The 10-pound sample obtained as prescribed in section 4.2 must be ground to completely pass through a No. 20 sieve (1 mm opening size). Use the Romer Mill - Model 2A, Viking Hammer Mill, or equivalent to grind the sample. Follow the manufacturer's instructions on how to operate the grinder. Observe all safety requirements as stated in chapter 3 of this handbook. Contact QCTB or the FGIS Weighing and Equipment Branch, Washington, D.C., if additional instructions are needed.

4.5 GRINDING

A. ROMER MILL. The Romer Mill simultaneously grinds and subsamples at the rate of approximately 1 pound per minute. An adjustable restrictor door located above the collection chute varies the amount of ground sample allowed into the collection chute. Official personnel must adjust the grinder to obtain approximately 500 grams from a 10-pound sample.

Adjust the grinder by locating the first line (far left) etched on the restrictor door. Position the door approximately 1/3 of the way between the first and second line. For a 10-pound sample, approximately 500 grams will be collected through the collection chute. Once the grinder is adjusted to obtain the 500-gram sample, mark the location of the setting. To increase the sample size, move the restrictor door to the left.

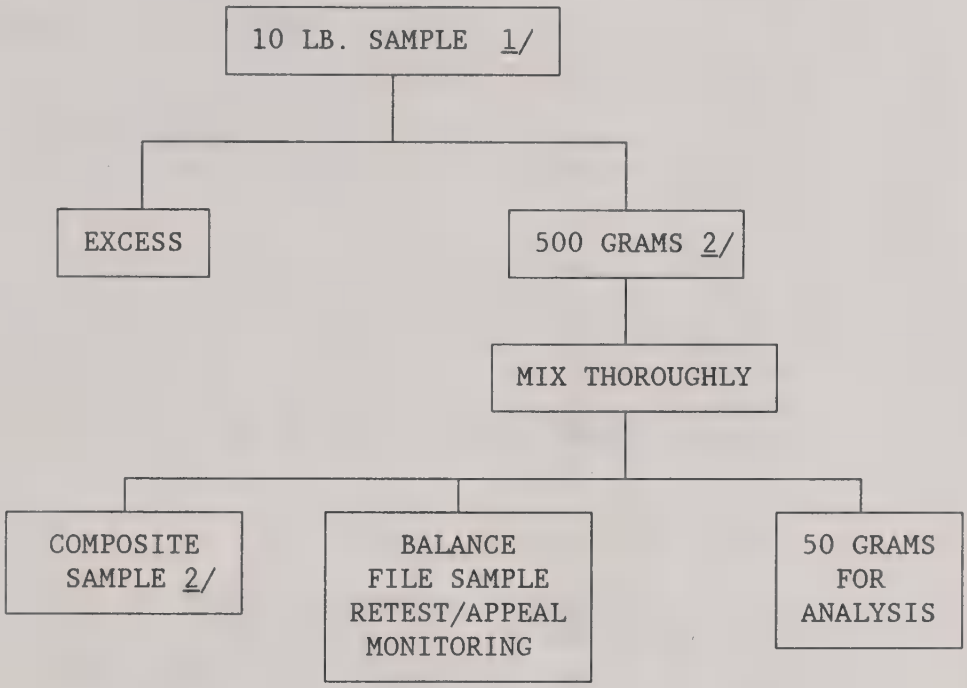
Samples with moisture content of 20 percent or more may cause the grinder motor to overheat and the breaker switch to release. If this occurs, allow the motor to cool and then set the grind lever to the coarsest setting by turning it counterclockwise. Do not grind high moisture samples on the fine grind setting.

1. Grinding the Sample. Grind the entire 10-pound sample with the grind lever set at the finest range.

Collect the 500-gram sample in a clean container and stir the sample with a clean spatula for about 30 seconds. Using a spoon or spatula dip out a 50-gram portion for analysis. Maintain the balance as a file sample.

If a composite sample is required in addition to the sublot-by-sublot analysis, adjust portion sizes as needed to obtain an adequate size composite and still maintain individual file samples. Obtain the composite sample from the ground sublot samples.

CHART NO. 1 - ROMER MILL



1/ For submitted samples, the amount submitted for testing.
2/ If a composite sample is required, adjust portion sizes as needed to obtain an adequate size composite sample and still maintain individual file samples.

2. Cleaning of Mill. A small amount of ground sample will remain in the mill after the total sample has been ground and a subsample collected. To prevent the contamination of subsequent samples, clean the mill using one of the following cleaning procedures:

a. If a vacuum cleaner is available. After a sample has been ground and collected, with the unit turned on, use a vacuum cleaner with an attachment that will fit over the mouth of the chute. Place the attachment at the bottom of each chute for about 30 seconds. After all three chutes have been cleaned, turn the power off and prepare for the next sample.

b. If a vacuum cleaner is not available. Clear the grinder by discarding a small portion (first 10 to 15 grams) of the next sample to be tested.

(1) Pour the sample into the grinder and turn it on long enough to collect the first 10 to 15 grams.

(2) Turn the power off, and discard the 10-15 grams ground sample.

(3) Turn the power back on and finish grinding the sample to collect the remaining subsample for analysis.

B. Viking Hammer Mill. The Viking Hammer Mill may be used for processing grains. Samples with moisture content of 20 percent or more may cause the grinder motor to overheat and the breaker switch to release. If this occurs, turn off the power switch and allow the machine to cool off. Push the reset button and turn the power back on.

1. Grinding the Sample.

a. For corn, coarsely grind the entire 10-pound sample using the 1/2-inch screen and collect the ground sample in a new polyethylene bag(s) or a clean plastic pail. For grains other than corn, contact QCTB or Weighing and Equipment Branch, Washington, D.C. to determine whether any special grinding procedures are needed.

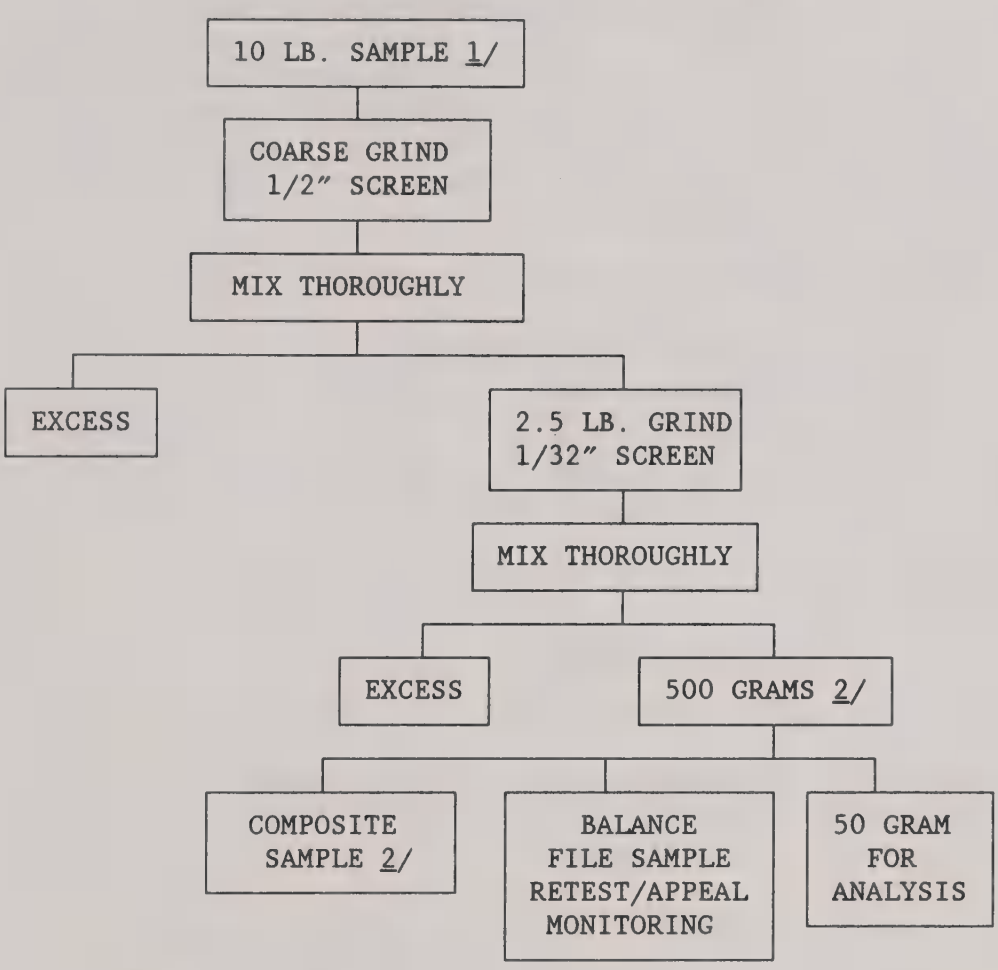
b. Using a riffle or similar type divider, cut out a representative 2.5-pound portion (approximately 1,135 grams).

c. Grind the 2.5-pound portion using the 1/32-inch screen.

d. Using a riffle or similar type divider, cut out a 500-gram sample from the 2.5-pound ground sample. From the 500-gram portion, cut out a 50-gram portion for analysis. Retain the balance as a file sample.

To prevent contamination, clean the grinder and the divider after each sample.

CHART NO. 2 - VIKING HAMMER MILL



1/ For submitted samples, the amount submitted for testing.
2/ If a composite sample is required, adjust portion sizes as needed to obtain an adequate size composite sample and still maintain individual file samples.

2. Cleaning of Mill.

a. After a sample has been ground and collected, turn off the mill and allow the blades to completely stop. Disconnect the electrical power or physically "lock out" the switch before cleaning or opening the cover. Open the cover and clean the mill and screen with a vacuum cleaner or brush.

b. Replace the screen and securely fasten the cover before beginning the next sample.

4.6 EQUIPMENT, SUPPLIES and SAFETY

A. EQUIPMENT

Romer Mill (or equivalent)

Viking Hammer Mill (or equivalent)

An FGIS approved balance

Riffle divider (size 50 x 24); Fisher Scientific No. 04-942D

File sample containers

Vacuum

B. SUPPLIES

Disposable weighing boats, large, 500 per package; American Scientific Products No. B2045-15

C. SAFETY ITEMS

Disposable fire-retardant laboratory coat

Disposable impermeable gloves

Dust masks, Moldex Model 2200 or 3M Model 8710

Hair protection; Lab Safety Supply EB 1357 or EB 1356

Safety glasses

4.7 FILE SAMPLE RETENTION

The term "file sample" means a representative sample or representative portion of a sample that is retained for a specific period of time.

Maintain a representative file sample of at least 450 grams for each lot, sublot, composite, or submitted sample tested. For submitted samples that are less than 450 grams, retain as large a sample as possible.

A. Sample Containers. Label each file sample with the test date and identification. Place the sample in paper bags or envelopes. Securely close the sample container to prevent cross-contamination by dust. Take precautions to ensure that file sample containers are strong enough to prevent loss of sample integrity when storing samples. Do not store samples near heat, windows or in direct sunlight. (Store samples in cold storage if available.)

B. Protection of File Samples. Store each file sample in a manner that will maintain the representativeness of the sample and prevent possible manipulation or substitution.

C. File Sample Retention Period. The retention periods required are prescribed in the Grain Inspection Handbook, Book II, Section 1.18, File Sample Retention (Grain); and FGIS Instruction 917-13, Uniform File Sample Retention System for Rice, Pulses, and Processed Products Inspected Under AMA.

D. Disposition of Samples. At the end of the retention period, label the file samples as follows: "FOR LABORATORY USE ONLY - NOT FOR USE AS FOOD OR FEEDSTUFF," and discard the file samples in a dumpster or landfill disposal site.

4.8 SHIPPING SAMPLES

When it is necessary to send samples to other laboratory locations, take precautions to maintain sample integrity by securely packaging the samples. Label the shipping container "NOT FOR HUMAN CONSUMPTION".

Refer to chapter 1 concerning the handling and mailing of appeal and Board appeal samples and chapter 16 concerning the handling and mailing of monitoring samples.

U.S. DEPARTMENT OF AGRICULTURE
Federal Grain Inspection Service
P.O. Box 96454
Washington, D.C. 20090-6454

CHAPTER 5

PROCEDURES FOR AFLATOXIN EXTRACTION

5.1 GENERAL INFORMATION

Prior to beginning the aflatoxin testing procedures, prepare the solutions required for testing. All of the test procedures described in this handbook, except for the TLC and the Neogen Agriscreen methods, require the same methanol extraction procedures to obtain the sample extract needed for analysis.

5.2 PREPARATION OF METHANOL SOLUTION

A. Chemicals Required. HPLC Grade Methanol and Deionized/Distilled Water.

B. To Prepare the 80% HPLC Grade Methanol Solution. Make up the solution by using the ratio of 8 parts HPLC grade methanol to 2 parts deionized/distilled water. Keep the solution in a bottle tightly capped when not in use. Label the 80 percent methanol solution bottle showing date of preparation. If the amount of the 80 percent methanol solution being prepared needs to be adjusted based on the workload at individual locations, make sure that the 8 parts methanol to 2 parts distilled/deionized water ratio is maintained.

Prepare the 80% HPLC grade methanol solution as follow:

1. Using a graduated cylinder, measure 2400 mL of HPLC grade methanol and place it into a clean carboy with spigot.

2. Add 600 mL of deionized/distilled water to the methanol and shake vigorously until it is completely mixed.

3. Label the container giving the mixture (80 percent methanol and 20 percent water), date of preparation, and initials of technician that prepared the solution.

4. Store this solution at room temperature in a tightly closed container until needed.

5.3 SAMPLE EXTRACTION PROCEDURES

A. Place a sheet of filter paper (Vicam 31240 or S&S 591 24 cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or a collection beaker.

B. Label the collection container with the sample identification.

C. Place the 50-gram ground sample test portion in a blender jar or container.

D. Add 100 mL of the 80 percent methanol solution to the blender jar or container.

E. Blend for exactly 1 minute at high speed.

F. Pour the resultant mixture into the funnel containing the filter paper and collect the filtered extract into the labeled collection container. Allow to drain entirely.

G. When the filtration is complete, remove the funnel and thoroughly mix the sample extract.

1. If a test tube was used to collect the extract, place a teflon coated cap on the test tube and shake the tube for approximately 10 seconds by hand or by using a Vortex mixer.

2. If a collection beaker was used to collect the extract, stir the sample extract with a clean stirrer for approximately 10 seconds.

Proceed to the applicable test procedures outlined in the following chapters of this handbook for analysis to be performed.

Filter approximately 50 mL of water through the filter containing the ground corn and allow to drain. Discard the filter paper and its contents (ground corn) into a plastic garbage bag for disposal. Dispose of the filtered wash in the solvent waste disposal container.

5.4 EQUIPMENT, SUPPLIES, AND SAFETY

A. EQUIPMENT AND SUPPLIES

Blender	Oster mixer, Model 848-31A; Oster Corp., or Waring Blender with S.S. blender container or similar.
Cutting Assembly	Process unit with sealing ring for Oster Mixer, Model 848-31A; Oster Corp. 937-45 or Eberbach blender jar or similar.

EQUIPMENT AND SUPPLIES (continued)

Bottom cap	Threaded for Oster Mixer, Model 848-31A; Oster Corp. No. 937-46
Square type jar	Designed to fit above.
Nalgene funnels	80 mm Top I.D., Stem 30 mm, Stem O.D. 18 mm; American Scientific Products No. F7465-2
Culture tubes	Disposable - 25 mm x 200 mm; with Teflon Lined Caps; American Scientific Products No. 14-915H
Test tube rack;	Capable of holding 25 mm x 200 mm culture tubes; 11-1/2' x 4-3/4" x 3-5/8"; American Scientific Products No. S9224-4
Filter paper	24 cm diameter; American Scientific Products No. F-2875-24
Cylinders	Polypropylene, graduated 1000 milliliter (mL) capacity 100 milliliter (mL) capacity
Carboy	Nalgene, polyethylene, with spigot, 2 gallon capacity; Fisher Scientific No. 02-963-6A
Vortex Mixer	American Scientific Products No. S822S (Optional)

B. SAFETY ITEMS

Disposable impermeable gloves

Disposable fire retardant laboratory coats

Chemical splash goggles

Storage cabinet, flammable materials

Fire extinguisher, dry powder

CHAPTER 6

AFLATEST PROCEDURES

6.1 AFLATEST MYCOTOXIN TESTING SYSTEM

A. General. The Aflatest Mycotoxin Testing System allows quantitative determination of total aflatoxins (B1, B2, G1, and G2) in parts per billion (ppb) or qualitative (screening) for aflatoxin in corn, sorghum, wheat, soybeans, rice, and processed products. The Aflatest method of testing for aflatoxin uses monoclonal antibody affinity chromatography.

Maintain an inventory system at each location to ensure that an adequate supply of chemicals is on hand to provide service.

B. Precautions.

1. Make sure the final filtered extract is transparent and free of particulates and insoluble materials.

2. When passing the specified sample extract through the Aflatest affinity column, use a slow and steady flow rate. Do not exceed two (2) drops per second.

3. It is important to collect all of the methanol sample eluate from the affinity column into the cuvette.

4. Make sure the cuvette is clean and avoid sample contamination.

5. Make sure the fluorometer is calibrated according to instructions.

6. Record fluorometer ppb readout after 1 minute.

7. If a sample result exceeds the 300 ppb level and the applicant requests quantitative results certified above the 300 ppb level, the test must be rerun as stated in section 6.6 before certificating the results. Otherwise, certify the results as "Aflatoxin exceeds 300 ppb."

8. If interference from radar, radio transmitters, or other sources occurs, contact the Weighing and Equipment Branch in Washington, DC, for further instructions.

9. When preparing solutions use clean glassware and pipetters.

10. Do not contaminate the stock bottle of concentrated developer. Keep the bottle tightly capped when not in use.

11. Label each stock bottle of concentrated developer with the date on which it was first opened. DO NOT USE IT IF 30 DAYS HAVE ELAPSED FROM THE DATE IT WAS FIRST OPENED.

12. Label each dilute developer solution bottle upon preparation showing date and time of preparation with an expiration time of 6 hours.

6.2 PREPARATION OF SOLUTIONS

Prior to beginning the procedures, prepare the solutions required for testing. The distilled/deionized water, dilute developer solution, and the HPLC grade methanol must be checked for background fluorescence with the fluorometer after the fluorometer has been calibrated. None of the above reagents should give a positive reading of more than 1.0 ppb.

A. Dilute Developer Solution - (TO BE PREPARED FRESH DAILY.) The concentrated developer solution should have a slight reddish brown color. (Do not use the stock solution if it is colorless. Loss of color indicates that the stock solution has lost its potency. Prepare dilute developer solution by adding 5 mL of Aflatest developer concentrate (VICAM Cat. # 32010) to 45 mL of distilled/ deionized water. Mix well. Label the dilute developer solution bottle showing DATE and TIME of preparation. DO NOT USE IT AFTER 6 HOURS HAVE ELAPSED. If the amount of dilute developer being prepared needs to be adjusted based on the workload at individual locations, make sure that the 1 part concentrated developer to 9 parts distilled/ deionized water ratio is maintained.

Label each stock bottle of concentrated developer with the date on which it was first opened. DO NOT USE IT AFTER 30 DAYS HAVE ELAPSED.

B. 80 Percent Methanol Solution. Make up the solution by using the ratio of 8 parts HPLC grade methanol to 2 parts deionized/distilled water. Prepare the 80 percent methanol solution by adding 800 mL methanol to 200 mL of water. Mix well. Keep the bottle tightly capped when not

in use. Label the 80 percent methanol solution bottle showing date of preparation. If the amount of the 80 percent methanol solution being prepared needs to be adjusted based on the workload at individual locations, make sure that the 8 parts HPLC grade methanol to 2 parts distilled/deionized water ratio is maintained.

6.3 SETUP FOR TESTING

Set up the pump stand with clamp attached.

Mount a clean 10 mL glass syringe barrel securely in the clamp.

Prepare an Aflatest-P affinity column for use by removing both end caps and shaking the buffer solution from the top of the column.

Place a waste collection cup under the affinity column.

6.4 CALIBRATION OF FLUOROMETER

A. General. The TorBex model FX-100 series-3 fluorometer is used to determine the aflatoxin level. To ensure accurate results, calibrate the fluorometer prior to use each day and verify at least once an hour using the YELLOW VIAL.

Turn the fluorometer on with the ON/OFF switch located on the rear panel. When the fluorometer is turned on, allow it to warm up for 10 minutes before calibrating. Once the fluorometer is turned on, it may be left on until close of business for the day. If the fluorometer is turned off during the day, a 10-minute warm up is required.

After turning the fluorometer on, it will identify itself and perform a set of self tests. The fluorometer will then proceed through a printer check. All self tests and the printer check must pass or an error message is displayed. If any error message appears, consult the operator's manual.

1. Date and Time. When date and time are first shown, the display reads "DATE XX/XX/XX TIME XX:XX:XX." Use keypad to enter the correct date and time as follows:

a. If date and time are correct, depress the arrow key under CONTINUE. Go to 2.

b. If date and/or time are incorrect, depress the arrow key under CHANGE. The display asks if date or time is to be changed. One is selected (date or time) and changes are made using the keypad. When the correct date and/or time are entered, depress the arrow key under ENTER. The system displays the corrected information. Depress the arrow key under CONTINUE.

2. Test Delay Time. The default test delay time is 60 seconds, which is correct for the Aflatest-P assay. Press ENTER.

3. Measurement Units. Set measurement units to ppb by pressing the arrow key under PPB. The fluorometer briefly displays "STARTING CALIBRATION, CHECKING GAINS, WAIT." Then the display message will change to "INSERT CALIBRATION VIAL."

B. Calibration Standards. The standard solutions in the three (3) standard vials (RED, GREEN, and YELLOW) degrade slowly in the presence of light. Since the plastic case containing the vials passes a small amount of light, it is recommended that both case and vials be stored in a cabinet or drawer away from all light except when calibrating or checking the calibration of the fluorometer.

Maintain two (2) sets of standards (two cases) at each location. Select and identify one set as the working standard, the other as the reference standard to be used to check the working standard every 14 days. The degradation of the working set will occur gradually over a period of time, so anticipate expiration and requisition a replacement set in advance. (A sudden change in the reading of a vial indicates instrument instability, a cracked vial, or undue exposure of the vial to light.) When one vial of a set expires, replace the entire set. About 2 months before the expected expiration of the working set, obtain a new set of standards from VICAM Co. On arrival, compare fluorometer readings of the new set with those of the existing reference set. If the difference between the two sets exceeds 3 ppb for any of the colors, notify QCTB.

Biweekly check of working standards. Calibrate the fluorometer using the working set as described in section 6.4, C. Then remove the reference set from storage and test the 3 vials as described in section 6.4, C, 8. The difference in readings of the two sets should not exceed the following limits:

Red (ppb)	Yellow (ppb)	Green (ppb)
± 10	± 5	± 2

If the difference between the working and reference sets exceeds the tolerances, discard the working set. Begin using the old reference set as the working set, and use the new set as the reference set. Keep a permanent record of all calibration verification data.

C. Calibration Procedures.

1. Turn on the fluorometer and warm it up for 10 minutes. Set date, time, test delay time, and measurement units as described in section 6.4, A. When the display shows "INSERT CALIBRATION VIAL," proceed with calibration as described below:

2. Wipe the RED VIAL with a clean cloth or paper wipe and insert it into the sample well. Be sure the vial is fully inserted and touches the bottom of the sample well. The display will read "CALIBRATOR VALUE 20.0 PPB PRESS ENTER." The correct value for the red vial is 150 ppb rather than the default value of 20 ppb. Enter the correct value of 150 ppb.

3. Use the keypad to enter 150, and depress ENTER to start the calibration sample measurement. The display will read "MEASURING CALIBRATOR." When the calibration measurement is completed, the display will read "REMOVE CALIBRATION VIAL."

4. After removing the RED VIAL, the display will read "INSERT BLANK VIAL, CONTINUE CALIBRATION." Wipe the GREEN VIAL with a clean cloth or paper wipe and then insert it into the sample well. Be sure the vial is fully inserted and touches the bottom of the sample well.

5. The display will read "BLANK VALUE 0.0 PPB, PRESS ENTER." The correct value for the green vial is -3 ppb rather than the default value of 0 ppb. Enter the correct value of -3 ppb. (The value of -3 is an offset to correct for a small amount of material which bleeds from the column during the assay.) Use the keypad to enter -3, and depress ENTER to start the blank measurement. The display will read "MEASURING BLANK." When the blank measurement is completed, the message will read "MEASURING BLANK, REMOVE BLANK VIAL."

6. After removing the GREEN VIAL, the display will read "PUSH ENTER TO CONTINUE, RECAL <SOFT KEYS> RETEST."

7. The calibration process is now completed. Depress ENTER to prepare the instrument for measuring unknown samples. The display will read "READY TO START TESTING, INSERT SAMPLE TO MEASURE."

8. Check the calibration by testing the YELLOW VIAL. Wipe the vial with a clean cloth or paper wipe and insert it into the sample well. Be sure the vial is fully inserted and touches the bottom of the sample well. The value displayed should be 75 $\pm$ 5 ppb. If it is not, repeat the calibration process (steps 2 through 7, above), then check the YELLOW VIAL again. To restart the recalibration process, depress the arrow on the left-hand side of the display. Record the result for the YELLOW VIAL in a permanent notebook. If the YELLOW VIAL still reads out of tolerance, contact the Moisture and AMA Testing Group at QCTB.

9. When the fluorometer is calibrated, place the standards back in the case and close the case.

D. Frequency of Calibration. Check the calibration of the fluorometer at least once an hour using the YELLOW VIAL.

6.5 TESTING PROCEDURES

A. Solution Testing. The distilled/deionized water, dilute developer solution, and HPLC grade methanol must be tested for background fluorescence before use. Calibrate the fluorometer as stated in section 6.4 and then perform the following:

1. Place 2.0 mL of HPLC grade methanol into a clean cuvette. Place the cuvette in the calibrated fluorometer. The displayed reading should be between -3.0 and +1.0. If the reading is positive and greater than 1.0, replace the methanol.

2. Dispense 2.0 mL of deionized/distilled water into a clean cuvette. Place the cuvette in the calibrated fluorometer. The digital display reading should be between -3.0 and +1.0. If the reading is positive and greater than 1.0, take action to assure a pure water supply.

3. Combine 1.0 mL of dilute developer solution and 1.0 mL of HPLC grade methanol in a clean cuvette. Place the cuvette in the calibrated fluorometer. The digital display reading should be between -3.0 and +1.0. If the reading is positive and greater than 1.0, check each reagent separately to determine which reagent is causing the problem and replace it.

B. Sample Extract. Prepare the sample extract as follows:

1. Place 50 g of ground sample into blender jar.

2. Add 5 g of analytical or USP grade NaCl.

3. Add 100 mL of the 80% methanol/water extraction solution.

4. Cover jar and blend at high speed for 1 minute.

5. Remove the cover and pour the extract into a filter paper (Vicam 31240 or S&S 591 24 cm pleated or equivalent) supported in a clean funnel.

6. Collect the filtrate in a clean beaker labeled with the sample identification.

7. After collecting approximately 25 mL of extract, carefully dispose of the filter paper and its contents as prescribed in Chapter 5.

8. Pipet 5 mL of filtered extract into a clean beaker.

9. Add 10 mL of distilled/deionized water and mix thoroughly.

10. Filter the diluted extract through a glass microfibre filter (VICAM Cat. # 31955) supported by a small, clean funnel. Fold the glass microfibre filter gently without making a sharp creased to avoid breaking the glass microfibre filter.

11. If this diluted filtrate turns cloudy, refilter using a new glass microfibre filter before proceeding with the analysis.

C. Analysis. Sample analysis using these procedures can be greatly simplified by the use of a small aquarium air pump to provide the needed air pressures for loading, filtering, and washing the various extracts.

1. TEST PROCEDURES FOR CORN, CORN MEAL, CORN/SOY BLEND, MILLED RICE, POPCORN, SORGHUM, AND SOYBEANS.

a. Prepare an Aflatest-P affinity column for use by removing both end caps and gently shaking the buffer solution from the top of the column.

b. Using an Eppendorf pipet, add 1.0 mL of the filtered dilute extract to the top of the Aflatest column.

c. Attach the column to the washing device (either a syringe barrel or an air pumping station) and pass the filtered extract through the column using a steady positive pressure. Maintain a flow rate not exceeding 2 drops per second.

d. After the extract has completely passed through the Aflatest column, add 1 mL of deionized or distilled water to the column and again apply a steady positive pressure to pass the wash water through the column. (If a syringe barrel rather than the pumping station is used, detach the column and pipet 1 mL of deionized or distilled water into the column headspace. Re-attach the column to the syringe barrel and apply pressure to pass the water through the column.)

e. Repeat the water wash in step d.

f. After the second wash has passed through the column, place a clean cuvette under the outlet of the column. Only 12 x 75 mm borosilicate glass tubes should be used for cuvettes (VICAM Cat. # 34000 or equivalent). Use care when handling the cuvette to keep the optical surface clean and free of lint, fingerprints, etc.

g. Dispense 1.0 mL of HPLC grade methanol into the column. If a syringe barrel rather than the pumping station is used, detach the column, pipet 1 mL of methanol into the column, and replace the column.

h. Apply a steady pressure to pass the methanol through the column and collect all of the methanol in the cuvette. Maintain pressure to collect the methanol at a rate of 2 drops per second or less.

i. Add 1.0 mL of dilute Aflatest Developer Solution directly to the sample solution in the cuvette and mix well (about 5 seconds).

j. Immediately place the cuvette in a calibrated TorBex Model FX-100 Series-3 Fluorometer.

k. Record the digital readout (at 60 seconds) as ppb total aflatoxins in the sample.

2. TEST PROCEDURES FOR CORN GERM MEAL AND WHEAT. The test procedure for corn germ meal and wheat is identical to that listed in sections 6.5, B and 6.5, C with the following exceptions:

a. Add 10 g (rather than 5 g) NaCl to the 50 g sample prior to extraction (step 2, section 6.5, B).

b. Use 200 mL (rather than 100 mL) of 80% methanol extraction solution (step 3, section 6.5, B).

c. After the extraction, if the solution filtration is slow (i.e. more than two minutes are required to collect 5 mL of filtrate), modify steps 8 and 9 (section 6.5, B) as follows: withdraw 5.0 mL of the clearest liquid from the top of the material held in the funnel and transfer it to a clean container. Add 10 mL of distilled/deionized water to the 5 mL aliquot and mix thoroughly. Pour the resultant mixture into the funnel containing the glass microfibre filter (step 10, section 6.5, B) and collect the filtered extract.

d. For the affinity column analysis, do steps b and c (Section 6.5, C, 1, b) twice. That is, two separate 1.0 mL portions of the diluted filtered extract should be loaded onto the Aflatest Affinity Column, and passed through the column separately.

3. TEST PROCEDURES FOR CORN GLUTEN MEAL. Using the Aflatest method, follow these procedures.

a. Weigh 50g ground sample and place in blender jar.

b. Add 5g NaCl to blender jar.

c. Add 250 mL of 60 percent methanol extraction solvent to blender jar (60% methanol extraction solvent prepared by mixing 600 mL HPLC grade methanol with 400 mL distilled/deionized water).

d. Cover jar and blend for one minute at high speed.

e. Remove cover from blender jar and pour a portion of mixture into fluted filter. Collect filtrate in clean vessel.

f. Using a 10 mL pipette, remove 10 mL of filtered extract and place into clean vessel.

g. Add 20 mL of distilled/deionized water to dilute the extract.

h. Mix diluted extract thoroughly.

i. Filter diluted extract through glass microfibre filter (VICAM #31955) and collect filtrate in clean vessel.

j. Load 6-8 mL of the filtrate from step i into a 10 mL plastic syringe barrel fitted with 0.22 micron nylon syringe disk filter. (Fisher Scientific Corporation CAMEO II Cat. No. DDN 02T2550, Gelman Cat. No. 09-730-191, or Corning Cat. No. 09-754-22).

k. Apply enough air pressure to syringe barrel to produce a flow of approximately 1 drop per second through disk filter and collect a minimum of 5 mL of filtrate in a clean test tube. Discard filter disk.

l. Using a 1.0 mL Eppendorf pipette, load 4.0 mL of refiltered extract from step k into the barrel of a 10 mL glass syringe to which an Aflatest-P column is attached.

m. Apply pressure so that the extract passes through the column at 1 to 2 drops per second. Remove syringe barrel from column. Fill column with distilled water. Re-attach syringe barrel to column.

n. Fill syringe barrel with 10 mL of distilled/deionized water and pass through column at a flow rate of approximately 2 drops per second. Allow all of wash water to pass through column.

o. Repeat column wash with another 10 mL of deionized/distilled water.

p. Elute aflatoxin from Aflatest-P column with 1 mL HPLC grade methanol and collect eluate in glass cuvette.

q. Calibrate fluorometer as follows:

RED VIAL	=	110	ppb
YELLOW VIAL	=	55 $\pm$ 5	ppb
GREEN VIAL	=	-3	ppb

r. Add 1 mL of fresh, dilute Aflatest developer solution directly to the eluate in cuvette and mix well.

s. Place cuvette in calibrated fluorometer and record reading after 60 seconds.

t. Rinse both glass and plastic syringe barrels with approximately 10 mL of distilled/deionized water each before analyzing next sample.

4. TEST PROCEDURES FOR RICE. All rice aflatoxin tests are performed on a milled rice basis. Consequently, rough rice or brown rice require milling. Mill rough rice or brown rice according to the procedures in the Rice Handbook, Chapters 3 and 4, respectively.

Prepare the milled rice sample for aflatoxin testing service according to the instructions in chapter 4.

Use the Aflatest method, Aflatoxin Handbook, chapter 6, section 6.5, C, 1 unless the applicant requested the TLC method.

6.6 SAMPLES THAT EXCEED 300 PPB

Procedures. To determine and report an aflatoxin level higher than 300 ppb:

1. Follow the instructions in section 6.5, except in step 6.5, C, 1, b, use 0.5 mL of the diluted filtered extract instead of 1 mL.

2. When 1 minute is elapsed and the digital reading from the fluorometer is obtained, multiply the reading by 2 to get the sample result in parts per billion.

(Example: 188 ppb x 2 = 376 ppb)

6.7 EQUIPMENT, SUPPLIES, AND CHEMICALS

EQUIPMENT	QUANTITY
TorBex Model FX-100 Series-3 Fluorometer	1
Fluorometer calibration standards - VICAM # 33050	2
Cuvette Rack - VICAM # 21010	1
Pump assembly stand, double - VICAM # 21030	1
Syringe, glass 10 mL - VICAM # 34010	2
Syringe hand pump with coupling - VICAM # 36030	2

Automatic pipetter (for methanol), 1 mL capacity - VICAM # 20501	1
Automatic pipetter (for developer), 1 mL capacity - VICAM # 20600	1
Graduated cylinder:	
25 mL capacity	1
100 mL capacity	1
250 mL capacity	1

SUPPLIES 1/

Aflatest-P columns - VICAM # 12022

Cuvettes, disposable 12 x 75 mm borosilicate glass tube - VICAM # 34000

Disposable beakers - VICAM # 36010

Glass microfibre filter paper (Whatman 934-AH) - VICAM # 31955

Small plastic funnels

Safety shears

Wash bottles or spray bottles

Box of Kim Wipes (small size sheets)

CHEMICALS 1/

HPLC grade methanol - VICAM # 35016

Aflatest developer solution - VICAM # 32010

1/ Maintain adequate inventory based on location workload.

Distilled/deionized water

Sodium chloride analytical or United States Pharmacopeia (USP) grade

SAFETY ITEMS

Disposable impermeable gloves

Disposable fire retardant laboratory coats

Chemical splash goggles

CHAPTER 7

AFLA-20 CUP PROCEDURES

7.1 ALFA-20 CUP TEST KIT

A. General. Afla-20 Cup test is an enzyme linked immunosorbent assay (ELISA) developed by International Diagnostic Systems Corporation. As the name implies, antibodies which react specifically with aflatoxins are contained in a cup.

B. Kit Contents.

1. Cups with Aflatoxin Antibody attached
2. Aflatoxin Enzyme, Dropper Bottle
3. Negative Control Solution, Dropper Bottle
4. Wash Solution, Dropper Bottle
5. Substrate A, Dropper Bottle
6. Substrate B, Dropper Bottle
7. Dilution Buffer for Samples, Translucent
8. Open Neck Bottle

7.2 TESTING PROCEDURES

A. Sample Extraction. Follow the instructions outlined in Chapter 5, section 5.3, to obtain the sample extract required for testing.

B. Analysis (for a 20 ppb cut-off).

Each day, before testing official samples, test at least one negative control cup to ensure that all reagents are functional. Perform the negative control test by applying two drops of the negative control solution to the center of a cup and two drops of the Enzyme. Let set for 1 minute before washing with 30 drops of the wash solution. Prepare substrate solution by mixing ten drops each of substrate A and B. Add the 20 drops of substrate solution to the cup. Let set for 1 minute, then read the result. A blue color indicates the reagents are functional. If the color remains white for at least 1 minute, then the reagents are not functional. Reagents must be replaced.

1. Using the 1.0 mL Eppendorf with a clean tip, transfer 200 microliters (uL) of dilution buffer to a culture tube (12 x 75 mm) and then add 100 uL of filtered extract.

2. Mix well and apply 100 uL to the center of the cup.

3. Allow the cup to set for 1 minute.

4. Mix equal amount of Substrate Solution A and Substrate Solution B. Do not combine Substrate Solution A and Substrate Solution B more than 10 minutes in advance of use.

5. Add 20 drops Substrate Solution mixture to each cup.

6. Allow the cup to set for 1 minute reaction time.

7. Interpretation of Test Results.

a. Less than or equal to 20 ppb:

The sample is considered less than or equal to 20 ppb when the cup color changes to blue.

b. Greater than 20 ppb:

The sample is considered greater than 20 ppb when the cup color remains white for at least 1 minute.

7.3 HIGHER CUT-OFF POINTS

The manufacturer recommends the following procedures to obtain higher cut-off points. If the operator wishes to use higher than 20 ppb cut-off points, the following dilution procedures can be used with the dilution buffer provided. Sample size remain the same as above.

Desired Sample Cut-off	Extract Volume Into Dilution Tubes	Dilution Buffer Used	Diluted Sample Onto Cup
30 ppb	100 uL	350 uL	100 uL
40 ppb	100 uL	500 uL	100 uL
50 ppb	100 uL	650 uL	100 uL
60 ppb	100 uL	800 uL	100 uL
70 ppb	100 uL	950 uL	100 uL
80 ppb	100 uL	1,100 uL	100 uL
90 ppb	100 uL	1,250 uL	100 uL
100 ppb	100 uL	1,400 uL	100 uL
200 ppb	100 uL	2,900 uL	100 uL
300 ppb	100 uL	4,400 uL	100 uL

The results are read the same way as above.

CHAPTER 8

E-Z SCREEN PROCEDURES

8.1 EZ-SCREEN TEST KIT

A. General. The EZ-Screen test is a sequential, competitive enzyme immunoassay. The kit is packaged in a small "blister pack" enclosed in a sealed foil pouch. Each kit has a shelf life of 6 months and is assigned a lot number and expiration date at production.

Record the lot number from the "foil pouch" that was used for each test on the worksheet or, when applicable, designate a space on the shiploading log and record the lot number along with results.

Maintain an inventory system at each location to ensure that an adequate supply of test kits is on hand in order to provide service upon request. To avoid needless disposal of unused, expired test kits, do not overstock the supply at any one location.

B. Kit Contents.

Quik-Cards	2
Enzyme	1 dropper tube
Negative Control	1 dropper tube
Substrate	1 dropper tube
Dilution Buffer	2 tubes of 2 ml each (screw cap)
Sample Pipettes	2 (DO NOT USE)

Confirm the expiration date of the test kit before using. DO NOT USE A KIT THAT HAS EXPIRED.

C. Precautions.

1. The kit in its original packaging can be used until the end of the month indicated on the label when stored under refrigeration from 36 to 48° F. DO NOT FREEZE any of the kit components. Do not expose reagents to temperatures greater than 95° F.

2. Remove kits from the refrigerator and allow them to stand at room temperature for a minimum of 1 hour prior to performing the test. Failure to allow test kits to reach room temperature prior to use may result in false positive results.

3. Do not open test kits until ready for use.

4. Do not allow test kits to remain out of the refrigerator for more than 8 hours. Return unopened kits to the refrigerator for further use. Establish procedures to ensure that unopened test kits are not repeatedly left at room temperature and returned to the refrigerator. Ideally, identify kits left from the previous day and use these first.

5. Do not use reagents from one kit lot number with reagents from a different kit lot number.

6. Do not use reagents after the expiration date.

7. Do not perform testing with more than two test cards at a time.

8.2 TESTING PROCEDURES

A. Sample Extraction. Follow the instructions outlined in chapter 5, section 5.3, to obtain the sample extract required for testing.

B. Analysis.

1. Select a test kit that has warmed to room temperature. Remove a 2 ml DILUTION BUFFER TUBE from the kit and write the sample identification on the tube. Transfer 1 ml of the methanol extract from the sample to be tested to the tube and replace the cap. Shake the dilution buffer tube to mix the sample extract thoroughly.

2. Using a felt tipped pen, label a test card with the sample number and the date. Do not mark the card closer than 1/2 inch from the ports. Do not touch the test sites labeled "CONTROL and SAMPLE."

3. Prepare the NEGATIVE CONTROL by removing the plastic shrink seal from around the dropper cap.

4. Prepare the ENZYME by squeezing the plastic dropper tube to crush the inner glass ampule. Use caution to ensure that the inner glass does not penetrate the outer plastic dropper tube. Tilt the tube back and forth for approximately 20 seconds to rehydrate and mix the contents. Do not shake vigorously or the contents will foam. Remove the plastic shrink seal from around the dropper cap.

5. Prepare the SUBSTRATE by squeezing the plastic dropper tube to crush the inner glass ampule. Shake the tube vigorously for approximately 20 seconds to rehydrate and mix the contents. Remove the plastic shrink seal from around the dropper cap.

6. Place the test card on a clean, flat surface.

7. Using a 50-ul pipette with a clean yellow tip attached, apply 50 ul of the diluted sample extract to the card site labeled SAMPLE. Do not touch the pipette tip to the port. Hold the pipette so that the tip is approximately 1/2 inch above the port and allow the drop to fall freely.

8. Apply one drop of NEGATIVE CONTROL to the "Control" port of the card. Do not touch the dropper tip to the port. Hold the tube so that the tip is approximately 1/2 inch above the port and allow drop to fall freely.

9. Allow the "Sample" and "Control" drops to absorb into the test ports before proceeding to the next step.

10. Hold the ENZYME tube with the tip downward. Dislodge trapped air bubbles by tapping the side of the tube or shaking the contents toward the tip. Discard the first drop of ENZYME and then apply 1 drop to both of the ports on the test card. Do not touch the dropper tip to the ports. Hold the tube so that the tip is approximate 1/2 inch above the ports and allow drops to fall freely.

11. Allow the ENZYME drops to absorb into the test ports before proceeding to the next step.

12. Apply 1 drop of NEGATIVE CONTROL to both ports on the test card. Do not touch the dropper tip to the ports. Hold the tube so that the tip is approximately 1/2 inch above the ports and allow drops to fall freely.

13. Allow the drops to absorb into the test ports before proceeding to the next step.

14. Carefully remove the excess liquid from around the edge of both ports on the test card using a cotton swab or clean, absorbent tissue. DO NOT TOUCH THE PORTS DIRECTLY.

15. Hold the SUBSTRATE tube with the tip downward. Dislodge trapped air bubbles by tapping the side of the tube or shaking the contents toward the tip. Discard the first drop of SUBSTRATE and then apply 2 drops to both of the ports on the test card. Do not touch the dropper tip to the ports. Hold the tube so that the tip is approximately 1/2 inch above the ports and allow drops to fall freely. Immediately set a timer for 5 minutes.

16. When the timer goes off, read the test results as follows:

a. If there is excess liquid at the edge of the ports, carefully remove it with a cotton swab or clean, absorbent tissue prior to reading.

b. If color is visible in both the "Control" and "Sample" ports before the end of the 5 minutes, the test may be read before the timer goes off.

c. Using adequate lighting, examine the test card while leaving it flat on the work surface.

d. The "Control" port must function properly in order to have a valid test result. If the "Control" port does not develop a readily detectable color (remains colorless), the test result is NOT VALID. Do not continue, repeat the entire procedure or contact QARD for assistance.

If the "Control" port develops a readily detectable color of grey-blue or blue, the test result is VALID. Proceed with interpretation of test sample.

17. Interpret the test results as follows:

a. Less than or equal to 20 ppb.

The sample is considered less than or equal to 20 ppb when the "Sample" port develops color (light grey, grey-blue or blue) over the surface of the port. Even though the "Sample" port may not yield a color equivalent to the color of the "Control" port, the sample is still considered less than or equal to 20 ppb.

b. Greater than 20 ppb.

The sample is considered greater than 20 ppb when the "Sample" port fails to develop color (remains colorless). If the "Sample" port develops a narrow band of light grey color at the edge of the port with the rest of the port remaining colorless, such samples are considered greater than 20 ppb.

8.3 EQUIPMENT AND SUPPLIES

EQUIPMENT/SUPPLIES 1/

EZ-Screen test kits

Timer (5-minute capacity)

Eppendorf pipette (or equivalent)
50 ul capacity

Eppendorf pipette tips, yellow
(or equivalent)

Felt tipped pens

Cotton swabs

Refrigerator

SAFETY ITEMS

Disposable, impermeable gloves

Disposable fire retardant laboratory coats

Chemical splash goggles

Fume Hood

Eyewash fountain

1/ Maintain adequate inventory based on location workload.

CHAPTER 9

SAM-A PROCEDURES

Section Number	Section	Page Number

[RESERVED]

U.S. DEPARTMENT OF AGRICULTURE
Federal Grain Inspection Service
P.O. Box 96454
Washington, D.C. 20090-6454

CHAPTER 10

OXOID PROCEDURES

Section Number	Section	Page Number

[RESERVED]

U.S. DEPARTMENT OF AGRICULTURE
Federal Grain Inspection Service
P.O. Box 96454
Washington, D.C. 20090-6454

CHAPTER 11

AGRI-SCREEN PROCEDURES

11.1 AGRI-SCREEN TEST KIT

A. General. The Agri-Screen test is a quick, diagnostic tool to predict the presence of aflatoxin in corn. The test is an enzyme immunoassay that provides easy-to-read visual color results. The kit is packaged in a sealed "cellophane bag" with a label indicating the lot number and expiration date.

Record the lot number from the "cellophane bag" that was used for each test on the worksheet or, when applicable, designate a space on the shiploading log and record the lot number along with results.

Maintain an inventory system at each location to ensure that an adequate supply of test kits is on hand in order to provide requested service. To avoid needless disposal of unused, expired kits, do not overstock the supply at any one location.

B. Kit Contents. The following items are packaged in a sealed cellophane package and are the components that make up the test kit.

1. Foil Pouch with:	
Aflatoxin Antibody-coated well strips	2
Red Marked Mixing Well Strips	2
2. Foil Pouch Substrate Pack with:	
Green-capped dropper bottle (H_2O_2)	1
Small green-capped tubes (TMB)	12
3. Foil Pouch Pipette Tip Pack with:	
Pre-set syringe	1
Pipette tips	60*

*For FGIS purchases, the manufacturer will provide additional pipette tips in a separate package.

4. Cellophane package with:	
Large blue-marked squeeze tubes (Hydration solution)	2
5. Cellophane package with:	
Blue-labeled bottles enzyme conjugate	2
Red-labeled bottle red stopping reagent	1
White-labeled bottle 20 ppb control	1
6. Separate envelope packaged with:	
Whirlpaks (for sample extraction)	12

C. Precautions.

1. Confirm the expiration date of the test kit before using. DO NOT USE A KIT THAT HAS EXPIRED.

2. Each kit comes as a complete package. After all antibody wells have been used, discard entire contents of package.

3. Do not use reagents from one kit lot number with reagents from a different kit lot number, (well strips, hydration solution squeeze tubes, enzyme conjugate, red stopping reagent, 20 ppb control, contents of H202 dropper bottle or green-capped tubes containing TMB). The spring syringe and unused pipette tips may be kept and used with other kits.

4. Store all test kit components in refrigerator when not in use.

5. Remove test kits from the refrigerator and allow them to stand at room temperature (68-72° F) for a minimum of 1 hour before use.

11.2 PRE-EXTRACTION PREPARATION OF CHEMICALS

This test requires aflatoxin extraction from the sample with a 55 percent methanol solution.

A. Chemicals Required. HPLC grade methanol and deionized or distilled water (55 percent methanol and 45 percent water).

B. Preparation.

1. Using a graduated cylinder, measure 550 mL of HPLC grade methanol and place it into a clean carboy with spigot.

2. Add 450 mL deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

3. Label the container stating the mixture (55 percent methanol and 45 percent water), date of preparation, and initials of technician that prepared the solution.

4. Store this solution at room temperature in a tightly closed container until needed.

NOTE: You may prepare larger amounts of solution if you expect a heavy workload as follows:

1100 ml HPLC grade methanol
900 ml deionized or distilled water

OR

1650 ml HPLC grade methanol
1350 ml deionized or distilled water

11.3 SAMPLE EXTRACTION PROCEDURES

A. Place a sheet of filter paper (Whatman 2V folded or S&S 24 cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or a collection beaker.

B. Label the collection container with the sample identification.

C. Place the 50-gram portion of the ground sample in the whirlpak provided with the kit.

D. Pour in 250 mL of the 55 percent methanol/water solution and securely close the whirlpak.

E. Shake the corn and methanol mixture vigorously by hand for 3 minutes.

F. Pour a small amount of extract from the bag into the filter paper mounted over the collection container.

G. Close bag securely and save extract until ready for disposal. Dispose of the remaining sample extract by either filtering out the liquid and separating it from the ground portion or pouring the entire mixture into the waste disposal container. This will vary depending on the requirements setup at each individual location to meet local, State and Federal laws.

11.4 ANALYSIS

A. <u>Preparation of Solutions</u>.

1. Tear off aluminum seal from one of the blue-labeled bottles and remove rubber stopper. Cut the

tip off one of the large blue marked squeeze tubes. Squeeze the contents of the squeeze tube into the bottle. Replace the stopper and swirl contents until the pellet has dissolved. Indicate on the bottle the date of preparation. DO NOT USE IT AFTER 7 DAYS FROM DATE OF PREPARATION. Keep refrigerated when not in use. Save the second blue-labeled bottle in refrigerator until needed. When needed, mix the second blue-labeled bottle in the same manner as above. Keep all solutions in refrigerator when not in use.

2. Prepare the substrate solution as follow:

a. Select a green-capped tube and place it in a holder so that the solution in the tube is not in the light. (These tubes are sensitive to the light. Check the tube prior to use to ensure that there is no blue color. If there is blue color, discard and select another tube.)

b. Squeeze 4 drops from the green-capped dropper bottle into the green-capped tube. The green-capped dropper bottle have sufficient materials to prepare six tubes of substrate solutions.

c. Invert the tube three times to mix. Substrate solution will last for 1 day if kept in the dark. (Remake the substrate when it begins to turn blue.)

3. Tear off aluminum seals from red-labeled and white-labeled bottles and set aside.

B. Test Procedures. When performing the following test, do not run more than six wells at a time.

1. Check the humidity indicator in the foil pouch containing the well strips before beginning the test to make sure there is no pink color present on the indicator. (If there is a pink color indicated, do not use the kit.)

Remove the red-marked mixing well strip and break off the number of wells needed for each sample including one for each control. Place in well holder. Return unused strips to foil package along with the humidity indicator and close tightly.

2. Remove antibody-coated well strip and break off the same number of wells as above. Return unused strips to package along with the humidity indicator and tightly close the package opening.

Using a marker with permanent ink that will not wash off, mark one end of the mixing well strip and one end of the antibody-coated well strip as the control then label the sample wells in order to identify wells after washing.

3. Firmly place a pipette tip on the syringe and add one preset amount from the blue-labeled bottle to each red-marked mixing well. Discard tip.

A preset amount of liquid is the amount drawn into the pipette tip when the syringe plunger is depressed and then released slowly.

PERFORM STEPS 4-8 AS RAPIDLY AS POSSIBLE.

4. Remove the stopper from the white-labeled control bottle. Firmly place a new pipette tip on the syringe and add one preset amount from white-labeled control bottle to the first mixing well of the red-marked strip. Discard tip. Replace rubber stopper on control bottle.

5. Repeat step 4 for each additional control well.

6. Firmly place a new pipette tip on the syringe and add one preset amount from sample collection container to second well of red-marked mixing strip. Discard tip.

7. Repeat step 6 for each additional sample, using separate well and new pipette tip for each.

8. Using a new pipette tip each time, mix sample by placing the tip in the sample and depressing the plunger 2 times, then quickly transfer a preset amount from the red-marked well of each sample to the corresponding antibody-coated well.

9. Wait 5 minutes. (Start timer after filling last antibody-coated well).

10. Initial reaction is now completed. Shake out the contents of antibody-coated wells. Using a wash bottle filled with distilled water, fill each antibody-coated well with water and shake out. Repeat 10 times. Remove all water droplets by turning wells upside down and vigorously tapping wells on paper towel.

10. Firmly place a new pipette tip on syringe and add one preset amount from green-capped tube to each antibody-coated well. Discard tip.

11. Wait 5 minutes.

12. Firmly place a new pipette tip on syringe and add one preset amount from red-labeled bottle to each well. Discard the pipette tip.

13. With a new pipette tip mix the solution in each well thoroughly by depressing the plunger 3 times to prevent layering. CAUTION: Use a new pipette tip when mixing each well to prevent cross contamination of samples.

C. Interpretation of Results. Place the well strip on a white surface when determining the results. Interpret the results as follows:

1. Less than or equal to 20 ppb:

The sample is considered less than or equal to 20 ppb if the sample well is darker (bluer) or the same as the control well.

2. Greater than 20 ppb:

The sample is considered greater than 20 ppb if the sample well is lighter (redder) than the control well.

Record the results on the work record and report results on the certificate.

11.5 EQUIPMENT, SUPPLIES, AND CHEMICALS

EQUIPMENT/SUPPLIES 1/

Collection beakers or test tubes

Filter paper

Funnels

Agri-Screen test kits

Timer (5-minute capacity)

Refrigerator

1/ Maintain adequate inventory based on location workload.

EQUIPMENT/SUPPLIES (continued) 1/

Markers (permanent ink that will not wash off)

Kim wipes or paper towels

Graduated cylinder:
250 ml capacity
1000 ml capacity

CHEMICALS

HPLC grade methanol

Deionized or distilled water

SAFETY ITEMS

Disposable, impermeable gloves

Disposable fire retardant laboratory coats

Chemical splash goggles

Fume Hood

Eyewash fountain

1/ Maintain adequate inventory based on location workload.

CHAPTER 12

MINICOLUMN PROCEDURES

12.1 PREPARATION OF MINICOLUMNS

Prepared minicolumns (MC's) are available from the Mycolab Company, P.O. Box 321, Chesterfield, MO, 63017. FGIS laboratories will prepare columns onsite.

The packing materials required are; (1) Florisil 100-200 mesh, (2) Silica gel-E. Merck No. 7734 Silica Gel 60 for column chromatography (3) Alumina Neutral-Brockman activity I, 80-200 mesh.

A. Place a small plug of glass wool or a glass bead in the bottom of a clean glass column to a depth of 5 mm. (Negative empty Mycolab columns may be reused to prepare fresh MC's once they have been washed and dried. Dispose of those which have positive results.)

B. With the aid of a funnel,

1. Using a small scoop, add sodium sulfate to a depth approximately 5-7 mm.

2. Using the same small scoop, add florisil to a depth of 5-7 mm. Tamp tube to be sure additions are level.

3. Using a larger scoop, add 2 scoops of silica gel to a depth of approximately 20 mm.

4. Using the small scoop again, add neutral alumina to a depth of approximately 10 mm.

5. Using the small scoop, add 5-7 mm of sodium sulfate.

C. If you prepare extra MC's, store them in an upright position in a vapor tight container containing a layer of Drierite.

12.2 PREPARATION OF SOLUTIONS

A. Salt Solution. Using the 1/2-gallon polypropylene bottle, dissolve 225 grams of sodium chloride and 225 grams of zinc acetate in 1500 ml of distilled water. Add 5.6 ml of glacial acetic acid to this solution using the 50 ml graduated cylinder. Shake well. Label: "Salt Solution," preparation date, and initials of technician preparing the solution.

B. Chloroform - Acetone Solution. Pour 225 ml of chloroform and 25 ml of acetone into one 1000-ml polypropylene storage bottle. Shake well. Label: "Chloroform-Acetone Solution," preparation date, and initials of technician preparing the solution.

C. Standard Aflatoxin Solution. The aflatoxin standard solution is prepared by the FGIS Quality Assurance and Research Division (QARD), USDA/FGIS, Technical Center, P.O. Box 20285, Kansas City, MO, 64195. Store the standard solution in the refrigerator until ready for use. Upon receipt of the vial and after each use, place a mark on the standard solution container at the point where maximum concaveness occurs.

12.3 TESTING PROCEDURES

A. Sample Extraction. Follow the instructions outlined in chapter 5, section 5.3, to obtain the sample extract required for testing.

B. Analysis.

1. Add exactly 15 ml of water measured in a graduated cylinder to a culture tube and mark the level. Add an additional 15 ml of water to the same tube and mark the 30-ml level. Pour out the water and mark additional culture tubes to be used in a day at the exact same height to indicate the 15-ml and 30-ml marks.

2. Add 15 ml of the sample extract to a premarked culture tube.

3. Add 15 ml of salt solution to the 15 ml of sample extract (up to the premarked 30-ml mark).

4. Shake the tube and contents vigorously for 10 seconds by hand or by using the Vortex-Genie mixer. To be properly mixed, the solution should form a clearly visible vortex the entire depth of the solution.

5. Filter the solution, after mixing, into a second culture tube using filter paper and funnel. Collect 15 ml of the filtrate (up to the first mark on the tube). Remove the funnel with filter paper.

6. Add 3 ml of toluene using an Eppendorf pipet. This will require three transfers at 1 ml each.

7. Shake the solution in the tube gently for 10 seconds using a swirling motion. Make sure the toluene is mixed with the entire solution. Let the layers separate.

8. Place a prepared MC in a support rack with a glass vial to catch the waste solvent. (If using MC's with cotton plug and wire in one end, remove before using.)

9. Pipet 1 ml of the top toluene layer of the culture tube into a prepared MC using the Eppendorf pipet with disposable tip. TAKE CARE TO DRAW ONLY FROM THE TOP LAYER OF THE CULTURE TUBE.

10. Let this extract drain into the MC and then add 3 ml of chloroform-acetone solution and let drain into the waste vial. Simultaneously, prepare a standard MC.

12.4 PREPARATION OF STANDARD MINICOLUMN

A. General. Before using the standard solution, check the solution level previously marked on the vial to make sure it is even with the mark on the vial. If it isn't, do not use the solution and contact QARD immediately.

When preparing the standard MC, place the standard solution under the hood and allow it to warm to room temperature before using.

Prepare a standard MC each day with the first MC performed that day. Prepare a new MC standard when performing additional MC tests during the day if there is a change in the fluorescent band at the top of the florisil layer.

B. Procedures.

1. Select a MC and support it by using a support rack to hold the column in an upright position. Use a glass vial to catch the waste solvent from the column.

2. Identify the MC as the standard. Add 1 ml of the standard solution to the column.

3. Allow the standard solution to drain into the column. Add 3 ml of chloroform-acetone solution and let drain.

4. Examine the sample MC and the standard MC with a ultra-violet lamp for a blue fluorescent band at the top of the florisil layer. Precautions: Examine on a nonglare surface, wear glass lens, safety glasses, or goggles, and do not look directly at UV bulbs.

a. If the band has no bluish tint, the sample is considered less than or equal to 20 ppb.

b. If the band is equal in intensity to the standard minicolumn band, the sample is equal to 20 ppb.

c. If the sample MC band is more intense than the standard MC, the sample is in excess of 20 ppb which is the actionable limits established by FDA.

Be careful, some samples containing no aflatoxin show a faint white, yellow, or brown fluorescent band at the top of the florisil layer.

Contact QARD, Research and Development Branch, immediately when there is a question regarding the intensity of the blue fluorescent band in the standard minicolumn. QARD will send a referee column for comparison.

12.5 MODIFIED MINICOLUMN PROCEDURES

This service is available on a request basis only. Applicants may request an aflatoxin analysis utilizing the procedure that indicates if the lot contains aflatoxin at levels greater than 10, 15, 100, 200, or 300 ppb.

A. Procedures for 10 ppb.

1. Using the Eppendorf pipet, transfer 1 ml of aflatoxin B1 standard solution provided by the QARD to a clean, dry, 8 ml vial.

2. Place a clean tip on the Eppendorf pipet, add 1 ml of fresh toluene to the same vial.

3. Place a teflon-coated cap onto the vial and shake it vigorously until the solution is well mixed (approximately 15 seconds).

4. Using the Eppendorf pipet, transfer 1 ml of this diluted standard solution into a clean MC. Allow it to drain into the column, then add 3 ml of chloroform/acetone solution. Save this column as the reference standard, label it 10 ppb "standard column."

5. Perform a MC test of the corn sample using the current MC procedure.

6. Using a UV lamp, compare the fluorescence of the MC from the sample tested with that of the "10 ppb standard" column (prepared in step 4 above). If the fluorescence of the sample column is equal to or less than that of the "10 ppb standard" column, the aflatoxin level does not exceed 10 ppb.

If the fluorescence of the sample column is greater than the "10 ppb standard" column, the aflatoxin level exceeds 10 ppb.

B. Procedures for 15 ppb.

1. Using the Eppendorf pipet, transfer 1 ml of aflatoxin B1 standard solution provided by QARD to a clean, dry 8 ml vial.

2. Using an adjustable pipet (at least 350 ul capacity), add 333 ul of fresh toluene to the same vial.

3. Place a teflon-coated screw cap onto the vial and shake vigorously until the solution is well mixed (approximately 15 seconds).

4. Using an Eppendorf pipet, transfer 1 ml of this diluted standard solution into a clean, freshly prepared MC. Allow to drain.

5. Add 3 ml of chloroform acetone 9:1.

6. Allow to drain.

7. Check under UV lamp for a blue fluorescent band at the top of the florisil layer. If the fluorescence of the sample column is equal to or less than the "15 ppb standard" column, the aflatoxin level does not exceed 15 ppb.

C. Procedures for 100, 200, and 300 ppb. Follow the procedures when testing to the normal 20 ppb level. These procedures apply when testing to 100, 200, and 300 ppb.

1. Label three clean, dry, 8 ml vials No. 1, No. 2, and No. 3. Place them in the vial rack.

2. Using the 1,000 microliter (1 ml) Eppendorf pipet, transfer 1 ml of the toluene extract from the top layer remaining in the test tube from the original analysis into vial No. 1.

a. Using a clean tip on the Eppendorf pipet, add 4 ml of fresh toluene to this vial. (This can be accomplished by placing several milliliters of toluene in a small clean glass beaker then making four transfers with the 1 ml Eppendorf pipet.) Cap the vial with a teflon lined cap, shake vigorously until the solution is well mixed (approximately 15 seconds).

b. Using a clean tip on the Eppendorf pipet, transfer 1 ml of the solution from vial No. 1 to a clean MC. Allow the solution to drain into the MC then add 3 ml of chloroform/acetone solution.

c. Using a UV lamp, compare the fluorescence of this MC with the "20 ppb standard" MC. If the fluorescence of the sample MC is equal to or less than the "20 ppb standard" MC, the aflatoxin level exceeds 20 ppb but does not exceed 100 ppb.

If the fluorescence of the sample MC is greater than the "20 ppb standard" MC, proceed to Step 3.

3. Using a clean tip on the Eppendorf pipet, transfer 1 ml of the solution from vial No. 1 to the vial labeled No. 2. Replace the cap on vial No. 1 to avoid evaporation of the sample and set aside.

a. With a clean tip on the Eppendorf pipet, add 1 ml of fresh toluene to vial No. 2. Cap the vial with a teflon lined cap and shake vigorously until the solution is well mixed (approximately 15 seconds).

b. Using a clean tip on the Eppendorf pipet, add 1 ml from vial No. 2 to a new MC. Allow the toluene solution to drain into the MC then add 3 ml of chloroform/acetone solution.

c. Using a UV lamp, compare the fluorescence of this MC with that of the "20 ppb standard" MC. If the fluorescence of the sample MC is equal to or less than the "20 ppb standard" MC, the aflatoxin level exceeds 100 ppb but does not exceed 200 ppb.

If the fluorescence of the sample MC is greater than the "20 ppb standard" MC, proceed to Step 4.

4. Using a clean tip on the Eppendorf pipet, transfer 1 ml of the solution from vial No. 1 to vial No. 3.

a. Using a clean tip on the Eppendorf pipet, add 2 ml of fresh toluene to vial No. 3, cap the vial with a teflon lined cap, and shake vigorously until the solution is well mixed (approximately 15 seconds).

b. With a clean tip on the Eppendorf pipet, add 1 ml of the solution from vial No. 3 to a new MC. Allow the solution to drain into the MC and then add 3 ml of chloroform/acetone solution.

c. Using a UV lamp, compare the fluorescence of this MC with the "20 ppb standard" MC. If the fluorescence of the sample MC is equal to or less than the "20 ppb standard" MC, the aflatoxin level exceeds 200 ppb but does not exceed 300 ppb.

If the fluorescence of the sample MC is greater than the "20 ppb standard" MC, the aflatoxin exceeds 300 ppb basis minicolumn method.

When the modified MC method is requested, there is no additional charge. The standard charge of $18.90 applies for the entire test.

12.6 EQUIPMENT, SUPPLIES, AND CHEMICALS

EQUIPMENT	QUANTITY
Ultraviolet lamp viewing cabinet	1
Test tube rack; capable of holding 25 mm x 200 mm culture tubes; 11-1/2" x 4-3/4" x 3-5/8"; American Scientific Products No. S9224-4	2
Cylinders, polypropylene, graduated	
50 milliliter capacity	1
100 milliliter capacity	2
250 milliliter capacity	1
1000 milliliter capacity	1
Vortex mixer; American Scientific Products No. S822S	1
Eppendorf pipet, 1000 microliter; American Scientific Products No. P5062-IL	1
Minicolumn stand; Mycolab No. 751; Mycolab Company P. O. Box 321 Chesterfield, Missouri 63017	1
Refrigerator	

SUPPLIES 1/

Nalgene funnels - 80 mm Top I.D., Stem 30 mm Stem O.D. = 18 mm;
American Scientific Products No. F7465-2

1/ Maintain adequate inventory based on location workload.

Culture tubes - Disposable - 25 mm x 200 mm; 196 per case; with Teflon Lines Caps; American Scientific Products No.14-915H

Folded filter paper, 24 cm diameter; 100 per box; American Scientific Products No. F-2875-24

Disposable weighing boats; large; 500 per package; American Scientific Products No. B2045-15

Vials with teflon-lined caps - 8 ml

Vials, Kimble (size 21 mm x 50 mm), 36 per box; American Scientific No. B7811-3

Disposable pipet-tips for Eppendorf pipet; 1000 per box; American Scientific Products No. P5059-801

Jar, 1 polyethylene, 2-liter capacity; Fisher Scientific No. 11-815-11A (Apply a layer of Drierite to cover the bottom of the jar. Keep unused prepared columns in this covered jar. Change the Drierite every 2 weeks.)

Glass wool, fiber; American Scientific Products No. G 6010

Borosilicate std wall tubing ca 6(id) x 190 mm tapered at 1 end to ca 2mm

Prepared minicolumns; 25 per package; Mycolab No. 201; Mycolab Company

8 ml vials; VWR Scientific No. 66011-824 case of 200 Teflon lined caps VWR Scientific No. 66012-372

1/ Maintain adequate inventory based on location workload.

CHEMICALS 1/

Acetic acid, glacial, reagent grade

Acetone, reagent grade

Chloroform, reagent grade

Distilled Water

Drierite ($CaSO_4$)

Florisil

Household bleach

Silica gel

Sodium chloride (reagent grade)

Sodium sulfate, granular

Toluene, reagent grade

Zinc acetate, reagent grade

Alumina, neutral, for column chromatography

Safety Items:

Disposable impermeable gloves

Disposable fire retardant laboratory coats;

Chemical splash goggles

Fume Hood

Eye Wash Fountain

1/ Maintain adequate inventory based on location workload.

U.S. DEPARTMENT OF AGRICULTURE
Federal Grain Inspection Service
P.O. Box 96454
Washington, D.C. 20090-6454

CHAPTER 13

THIN-LAYER CHROMATOGRAPHY PROCEDURES

13.1 SEPARATION AND ISOLATION OF AFLATOXINS

Procedures described are in accordance with the Official Methods of Analysis of the Association of Official Analytical Chemistry (AOAC), Fifteenth Edition, 1990, sections 972.26 and 968.22A-D, Aflatoxin in Corn, TLC, Final Action, 1988, AACC-AOAC method and sections 972.27 and 968.22, Aflatoxin in Soybeans, TLC, Final Action, 1988.

WHEN PERFORMING THE FOLLOWING PROCEDURES, AVOID EXPOSURE OF SAMPLES TO FLUORESCENT LIGHT. DO NOT CONDUCT AFLATOXIN SEPARATIONS AND TLC DETERMINATIONS UNDER FLUORESCENT LIGHT. CONDUCT ALL PROCEDURES UNDER A HOOD.

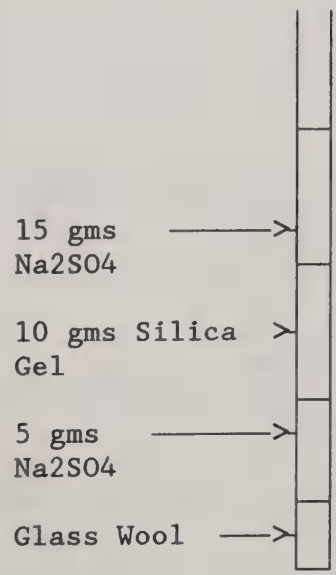
A. Weigh 50.0 grams of ground sample and place in 500-mL erlenmeyer flask.

B. Add 25 grams of diatomaceous earth, 25 mL distilled water, and 250 mL of chloroform (CHC13) to the ground sample.

C. Wrap stopper with foil and place securely on flask.

D. Shake contents in flask for 30 minutes on wrist-action shaker.

E. Prepare a column for separating aflatoxin as follows:



1. To a 250 mL chromatography column, place a glass wool ball near the stop-cock and add approximately 1 inch of chloroform.

2. Add 5 grams anhydrous Na2SO4 (sodium sulfate). Wash sides of tube with chloroform until the level of the tube is 1/2 full of chloroform.

3. Gently add 10 grams of silica gel (activated). Wash sides of tube with ca 20 mL chloroform. Stir to disperse silica gel. When rate of silica gel settling slows, drain chloroform into beaker until the level of chloroform is ca 5-7 cm above the gel layer.

4. Gently add 15 grams of anhydrous Na2SO4. Drain chloroform to the top of Na2SO4 layer.

F. Filter mixture through fluted filter paper. Collect the first 50 mL of sample extract and add to the prepared column. Drain sample extract to the Na_2SO_4 layer.

G. Add 150 mL hexane to the column and drain to the Na_2SO_4 layer. Discard eluted solvent.

H. Add 150 mL anhydrous ether (CAUTION: FLAMMABLE) to the column, and drain to the Na_2SO_4 layer. Discard eluted solvent.

I. Place a heat resistant glass collection beaker under the column. Add 150 mL $CHCl_3$:MeOH (Chloroform/Methanol, 97/3, v/v) to the column and collect the entire fraction in a beaker from time of addition of chloroform/methanol until flow stops.

J. Place 3-4 glass beads in the beaker and evaporate the solvent to near dryness (**using low heat**) so that approximately 1 mL of solvent remains in the beaker. DO NOT EVAPORATE TO COMPLETE DRYNESS.

K. Using an Eppendorf pipet, wash the inside walls of the beaker with approximately 1 mL of chloroform and transfer the contents into a glass vial.

L. Repeat step K two more times.

M. Evaporate contents in glass vial to dryness and cap until ready to begin spotting. Store in refrigerator unless spotting is to begin right away.

13.2 SPOTTING THE TLC PLATE

When spotting, always rinse microsyringe approximately 10 times with chloroform between spotting the standard solution and the samples.

If the sample has been stored in the refrigerator, remove the sample from the refrigerator and warm to room temperature under the hood before proceeding with spotting.

A. Condition TLC plates before use by heating in hot air oven, approximately 1 hour at 110° C. Cool plates to room temperature before use.

If additional plates are conditioned, store them in a air tight container until ready for use. If there is reason to think that the plates have picked up moisture since being conditioned or if 2 weeks have elapsed since being

conditioned, the unused plates can be reconditioned by repeating the above procedures.

B. Examine the TLC plate for flaws.

C. Score the silica gel by drawing a line across the TLC plate at 10 cm. This line will mark the distance the solvent moves during plate development.

D. Uncap vial, and add 200 uL of chloroform which represents the concentration of sample. Cap immediately, and shake vial to dissolve contents.

E. Rinse 10 uL syringe with chloroform.

F. Using a TLC template, spot 1, 3, 5, and 7 uL portions of aflatoxin standard on the TLC plate and record amounts and concentrations of standards. Spots should be small and uniform in size.

G. Rinse the syringe about 10 times with chloroform.

H. On the same TLC plate, spot 3 and 5 uL portions of the sample and record the sample number along with amounts and concentrations spotted. Spots should be small and uniform in size.

I. The spots should be free from solvent before being placed in the developing tank.

13.3 DEVELOPING THE TLC PLATE

Add the chloroform/acetone developing liquid (see section 13.8) to a TLC developing tank to approximately 1 cm. The level of the solvent should be below the level of the spots on the TLC plate.

The tank should be setting level and not moved during TLC development. To ensure uniform development of the plate, clean the tank each morning before adding the developing solution.

A. Carefully place the TLC plate in conditioned developing tank and cover with the glass cover.

B. Allow the solvent to migrate to the scored line at 10 cm.

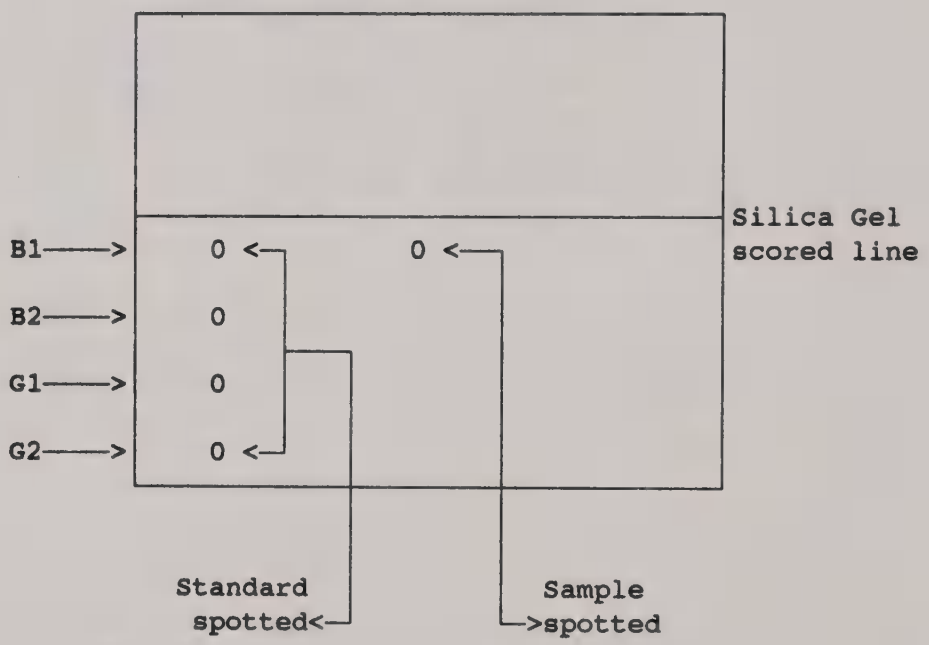
C. Remove the plate and place flat on counter in operating fume hood, to allow the solvent to evaporate. AVOID EXPOSURE OF PLATE TO FLUORESCENT LIGHT.

13.4 QUANTIFICATION OF AFLATOXIN IN SAMPLE

A. Illuminate the TLC plate with UV lamp. CAUTION: PROTECT YOUR EYES WITH UV-ABSORBING FILTER.

B. Observe the four fluorescent spots of the reference standards. From top to bottom, the Rf (distance spot moved/distance solvent moved) represent Aflatoxins B1, B2, G1, G2. Note the bluish fluorescence of "B" contrasted with slightly green "G" aflatoxins.

EXAMPLE OF DEVELOPED TLC PLATE UNDER UV LAMP



NOTE The standard solution displays four spots after developing. This is because the standard solution contains B1, B2, G1, and G2 differing compounds of aflatoxin. Typically only the B1 compound will appear in the sample spot.

C. Focus your attention to the intensity of aflatoxin B1 from the standard and compare with the intensity of aflatoxin B1 from the sample and attempt to find two B1 spots which match in intensity.

D. If aflatoxin B1 from the sample is more intense than aflatoxin B1 from the standards (indicates the concentration of B1 from the sample is too high), dilution of the sample extract is required. (See Section 13.6, Dilution for High Intensity Readings).

E. If the intensity of aflatoxin B1 from the sample can be matched with the intensity of aflatoxin B1 from the standard, proceed with the calculations in section 13.5 to determine quantitative aflatoxin concentration in sample.

13.5 CALCULATIONS

CALCULATIONS FOR DETERMINING AFLATOXIN B1 IN SAMPLE

Calculate the concentration of aflatoxin B1 in ug/kg (micrograms per kilogram) or ppb (parts per billion) using the following formula:

ug/kg or ppb = (S x Y x V)/ (X x W)

where: S = uL aflatoxin B1 standard equal in intensity to intensity of aflatoxin B1 in sample.

Y = Concentration of aflatoxin B1 standard in ug/mL. (1 ug/mL)

V = uL of final dilution of sample extract.

X = uL of sample extract spotted giving fluorescent intensity equal to S (B1 standard).

W = X gram sample applied to column if 50 mL of chloroform extract is used:

where: X = 10 gms for corn, rice, and other grains or

X = 9.6 gms for soybeans

Corn, Rice, and Other Grains calculate as:

$$\frac{\text{uL of std plated x conc of std ug/mL x 200 uL}}{\text{uL of sample plated x 10 gm}}$$

OR

Soybeans calculate as:

$$\frac{\text{uL of std plated x conc of std ug/mL x 200 uL}}{\text{uL of sample plated x 9.6 gm}}$$

13.6 DILUTION FOR HIGH INTENSITY READINGS

When the intensity of the sample is brighter than the standard spotted on the plate, it is necessary to dilute the sample extract and requantify as follows.

A. Evaporate the sample extract remaining in the glass vial to dryness on low heat on hot plate or steam bath. (If available, a stream of nitrogen gas applied to the surface of the sample will result in rapid evaporation of solvent.)

B. Cool vial and cap.

C. Add 400 uL of chloroform which represents the new concentration of the sample, cap vial, and shake to dissolve contents.

D. Repeat procedures for spotting the plate by spotting uL aliquots from the new dilution. Maintain the same solvent system in the developing tank. The other half of the TLC plate may be used for spotting and developing.

E. If further dilutions are necessary to determine aflatoxin B1 in the sample, follow the same procedures by first drying the contents in the sample vial, adding the selected volume of chloroform (600 uL or 800 uL), and repeating spotting procedures.

When calculating results insert the ul of final extract dilution into the formula as applicable, ex., 400, 600, or 800.

Corn, rice, and other grains calculate as:

$$\frac{\text{uL of std plated x conc of std ug/mL x 400 uL}}{\text{uL of sample plated x 10 gm}}$$

OR

Soybeans calculate as:

$$\frac{\text{uL of std plated x conc of std ug/mL x 400 uL}}{\text{uL of sample plated x 9.6 gm}}$$

13.7 PREPARATION OF SILICA GEL

Preparation of the silica gel for TLC testing is required. The silica gel must be dried to 1 percent moisture to properly activate the gel.

A. Weigh 105 to 110 grams of silica gel.

B. Place the silica gel in a porcelain drying dish.

C. Place the dish in the oven and bake at 105° C to 110° C for 1 hour.

D. Remove the dish from the oven, transfer the silica gel to a 500 mL flask and cap.

E. Allow the silica gel to cool to room temperature. Once the silica gel has cooled, perform the following steps rapidly but carefully.

F. Using a torsion balance scale weigh exactly 100 grams of the dried silica gel. (Discard the balance of the silica gel remaining from the 105-110 gram portion.)

G. Using a clean funnel, transfer the silica gel to a clean flask.

H. Add 1 milliliter of distilled water to the flask using an eppendorf pipet, place a cap on the flask.

I. Using one of the following methods, thoroughly mix the silica gel and distilled water.

1. Place flask on the "wrist action shaker" allowing it to shake for 1 hour.

2. Vigorously shake by hand for 1 minute. Repeat this step every 15 minutes for 1 hour.

J. Label the flask "Activated Silica Gel-1% H2O" and show the date and time of day.

K. Allow the prepared silica gel to sit for at least 15 hours.

L. After required time has elapsed, shake the flask by hand for approximately 10 seconds.

M. Weigh 10-gram portions into individual plastic vials and cap immediately. (Replace the cap on the flask when weighing portions).

If there is reason to think that the silica gel has increased in moisture content since being prepared or if 2 weeks have elapsed since it was activated, the unused silica gel can be reactivated by repeating the above procedures.

13.8 PREPARATION OF SOLUTIONS

Chloroform/Methanol Solution (97/3%)

Using a 1000-mL graduated cylinder, measure 970 mL of chloroform and transfer to the storage bottle with the aid of a funnel. Using a 50-or 100-mL graduated cylinder, measure 30 mL of methanol and transfer to the storage bottle containing the chloroform. Swirl to mix. LABEL THE BOTTLE.

Developing Liquid (88/12%)

Using a 100-mL graduated cylinder, measure 88 mL of chloroform and transfer to the storage bottle with the aid of funnel. Using a 50 mL graduated cylinder, measure 12 mL of acetone and transfer to the storage bottle containing the chloroform. Swirl to mix. LABEL THE BOTTLE.

TLC Standard Solution: Aflatoxin Mix-1 Kit

Upon receipt of package, check to see that there are five amber colored ampules enclosed. Examine the vials to see if the levels in each bottle are approximately the same. Store in the refrigerator until ready for use.

When ready to use, remove one vial of the standard from the refrigerator. Allow to set under the hood until contents reach room temperature.

Break the seal and transfer contents to a vial (2-5 mL) equipped with a teflon-coated screw cap that can be securely closed. Wrap vial with foil to avoid exposure to light. LABEL THE VIAL "TLC STANDARD" AND DATE PREPARED.

Always refrigerate when not in use and warm to room temperature before use.

13.9
CHEMICALS

Acetone, ASC grade
Celite 545
Chloroform, Reagent
Distilled Water
Ether, Anhydrous (≤ 0.01% alcohol)
Hexane
Methanol
Silica Gel 60, 0.063-0.2 mm (E.Merck Darmstadt) or equivalent
Sodium Sulfate, Anhydrous, Granular ACS grade
Aflatoxin Standard mix-1 kit

13.10
EQUIPMENT AND
SUPPLIES

Laboratory Equipment	CATALOG NO.	COMPANY
Glass Fiber (wool)	125-146	C
Glass beads-5mm	125-047	C
TLC plates (20 x 20 cm (8 x 8"))	255-851	C
Spotting Template	066-704	C
Developing tank	067-561	C
Micro-Syringe 10 uL	222-950	C
and Replacement needles for 10 uL syringe		
Chromatographic columns (22 x 300 mm		
Reservoir type 250 mL with Teflon Stopcock	055-814	C
Wrist Action Shaker	205-468	C
Clamps for wrist action shaker		
Erlenmeyer flask-500 mL, rubber stoppered		
Eppendorf pipet, 200 uL	331-561	C
Pipet tips	382-515	C
Eppendorf pipet, 1000 mL	21-370M	F
Pipet tips	21-372	F
Beakers, stainless steel	028-597	C
Clamps	383-174	C
Weighing Boats, large		
Glass rods, 5mm OD	125-831	C
Filter paper, S&S #588, 24 cm	093-906	C
Funnels, 100 mm diam, stem 20 cm		
Graduated cylinders 50 mL, 100 mL, and 250 mL capacity		

Laboratory Equipment	CATALOG NO.	COMPANY
Vials, opticlear with polyethylene stoppers, 3 gm capacity		
Blue M Utility Oven with thermometer	13-258-30A	F
Porcelain evaporating dish	08-693F	F
500 2 mL erlenmeyer flask with screw cap	10-093-10D	F
Polyethylene vials with snap on caps	03-388-G	F
Hot plate		
Light viewing cabinet (Ultra-violet) Chromatavue	245-407	C
Grinder, Romer Mill-Model 2A or equivalent		
Refrigerator		
Fire Proof Solvent Storage Cabinet		
Riffle sampler		

Safety Equipment

Emergency Shower
Emergency Eye Wash Fountain
Fume Hood ducted to the outside
Goggles
Impermeable Gloves
Rubber Aprons

Note: Some of the aforementioned equipment (e.g., fume hood ducted to the outside) the field office already has as part of their minicolumn laboratory. It is not necessary that separate equipment be purchased for performing TLC analyses unless the space does not meet laboratory criteria.

C - Curtin Matheson. These catalog numbers are taken from an old catalog, they may have changed somewhat in the 1988 catalog.

F - Fisher. These catalog numbers are taken from the 1988 catalog.

U.S. DEPARTMENT OF AGRICULTURE
Federal Grain Inspection Service
P.O. Box 96454
Washington, D.C. 20090-6454

CHAPTER 14

WASTE DISPOSAL

14.1 GENERAL

Proper disposal of hazardous waste is required by law. The Environmental Protection Agency (EPA) establishes specific guidelines; however, additional local and State laws exist in some locations.

It is important that the procedures used for disposing of waste chemicals comply with the laws required at each location.

Contact the local EPA office for disposal information and names of certified waste disposal companies in the area. FGIS must contact the Field Servicing Office in Minneapolis to discuss contracting requirements and to establish a purchase contract.

14.2 WASTE DISPOSAL

A. Chemicals and Solvents.

1. Dispose of waste according to existing local, State, and Federal laws.

2. Select an EPA approved or certified waste disposal company in the area. The company must be able to identify the type of waste drum required, provide information regarding sample profile and waste manifest requirements, and provide estimated cost for pick up based on the results of the sample profile.

3. Locate waste drums in an area outside the laboratory space that complies with local fire and EPA codes. Label and date waste drums properly.

4. Post disposal procedures at each laboratory site.

5. Maintain accurate records with documentation from the disposal company of pick up and delivery of the waste drums to the waste disposal site.

B. Decontaminated Materials. Place decontaminated materials, such as filters, test kit components, and disposable lab materials into a double heavy duty garbage bag and dispose of to a dumpster or landfill disposal site. Only the materials that have been decontaminated may be transported. (Do not transport flammables or contaminated materials).

C. Other. Label excess ground corn/other grains remaining after aflatoxin testing and ground corn/other grains from official aflatoxin file samples representing grain with greater than 20 ppb: "FOR LABORATORY USE ONLY - NOT FOR USE AS FOOD OR FEEDSTUFF" and dispose of in a dumpster or landfill site.

CHAPTER 15

REPORTING RESULTS AND CERTIFICATION

15.1 CERTIFICATING TEST RESULTS

A. General. The type of service requested and the test method used determines how aflatoxin results are recorded and certificated.

For qualitative service (regardless of the method used), certificate results as being equal to or less than a threshold (e.g., 20 ppb) or as exceeding the threshold. If a quantitative method is used to provide qualitative service, record the quantitative test results on the laboratory work records to the nearest ppb even though the results are certificated as meeting or exceeding a threshold.

For quantitative service, record results on the work record to the nearest ppb. If an applicant specifically requests aflatoxin certification at a level lower than 5 ppb and the Aflatest method is used for determination, record results on the work record to the nearest ppb. However, certificate only results equal to or greater than 5 ppb to the nearest ppb. Certificate results less than 5 ppb as "does not exceed 5 ppb." Quantitative certification of results less than 5 ppb is permitted on a case-by-case basis.

When test results indicate no presence of aflatoxin, certificate results as "not detected" or the phrase "The aflatoxin result is negative" or "Negative aflatoxin" may precede the applicable statement listed in section 15.5.

B. Grain (USGSA). All grains for which a standard has been established are tested for aflatoxin under the authority of the United States Grain Standards Act (USGSA). Under USGSA, the aflatoxin results are recorded on a pan ticket, worksheet, or log and in the "Remarks" section of the official certificate.

Sections 800.125 and 800.135 of the regulations under the USGSA permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factors and official criteria may be handled separately even though both sets of results are reported on the same certificate. When official grade or official factors and official criteria are reported on the same certificate, the review inspection certificate shall show a statement indicating that the review results are for official grade, official factors, or official criteria and that all other results are those of the original, reinspection, or appeal inspection results, whichever is applicable.

Certificate grain aflatoxin test results in accordance with sections 800.160 through 800.166 of the regulations under USGSA. Applicants may request separate certificates for aflatoxin and grade when aflatoxin and grade are determined on the same lot. Refer to sections 1.10 and 1.11 of this handbook for specific information regarding additional information for the certification of unit trains and export shiplots.

C. Commodities (AMA).

Upon request, commodities are tested for aflatoxin under the authority of the Agricultural Marketing Act (AMA). Certificate test results in accordance with sections 68.52, 68.63, and 68.70 through 68.75 of the regulations under AMA and the provisions of this handbook. Refer to sections 1.10 and 1.11 of this handbook for specific information regarding additional information for the certification of unit trains and export shiplots.

D. Kinds of Certificates.

1. USGSA Certificates. Issue the appropriate kind of certificate according to the type of service requested. Certificates may differ based on the movement (export or domestic shipments) as well as the sample and testing basis (sample-lot, warehouseman's sample, or submitted sample). Refer to the Grain Inspection Handbook, book IV, chapter 3, to determine the appropriate kind of certificate.

2. AMA Certificates. Use one of the following certificates for certifying aflatoxin test results. Complete these certificates as shown in attachments 1 and 2.

a. Form FGIS-993, Commodity Inspection Certificate, for lot inspections.

b. Form FGIS-957, Commodity Inspection Certificate (Sample Inspection), for submitted sample inspections.

E. Issuing Certificates. When samples are sent to CTL by a field office, the CTL manager will notify the respective field office of the results and the field office will inform the applicant, issue the certificate, and bill accordingly. CTL bills for services and issues certificates when an applicant sends a submitted sample directly to CTL for testing.

When a field office is not equipped to perform the type of service requested and samples are sent to another FGIS laboratory for testing, the testing office will notify the applicant of the results, issue the certificate, and bill the applicant unless otherwise arranged by the involved field office.

15.2 REINSPECTION AND RETEST CERTIFICATES

A. General.

1. Report results on the same kind of certificate issued for the original service.

2. The reinspection/retest certificate supersedes the original inspection certificate. Enter the following statement on the reinspection/retest certificate:

"This certificate supersedes certificate No. (number) dated (date)."

3. The superseded certificate is null and void as of the date of the reinspection/retest certificate. Enter the following statement when the superseded certificate has not been returned at the time the reinspection/retest certificate is issued:

"The superseded certificate has not been surrendered."

4. When a file sample is used, enter the following statement on the reinspection/retest certificate:

"Results based on file sample."

B. Grain (USGSA). When a reinspection is requested, the laboratory that conducted the original analysis also conducts the reinspection using the official file sample unless a new sample is requested and permitted by the regulations. Any approved testing method available at the testing location may be used for the reinspection. However, if the original test results were based on a quantitative test, a quantitative method is also used for the reinspection.

In addition to the statements required in section 15.2, A:

1. Indicate "Reinspection" on the certificate.

2. When a new sample is used, enter the following statement:

"Results based on new sample."

3. When reporting more than one official result on the same certificate but at different levels of inspection, explain this condition using the following statement:

"(Grade, factor, or official criteria) results based on (new/file) sample. All other results are those of original inspection service."

C. Commodities (AMA). When a retest is requested, the laboratory that conducted the original analysis also conducts the retest using the official file sample. Any approved testing method available at the testing location may be used for the retest.

In addition to the statements required in section 15.2, A, type the word "Retest" in the Level of Inspection block on the FGIS-993 form or in the upper center of the original and each copy on the FGIS-957 form.

15.3 APPEAL CERTIFICATES

A. General.

1. Report results on the same kind of certificate issued for the original, reinspection, or retest service.

2. The appeal certificate supersedes the previous (original, reinspection, retest) certificate. Enter the following statement on the appeal certificate:

"This certificate supersedes certificate No. (number) dated (date)."

3. The superseded certificate is null and void as of the date of the appeal certificate. Enter the following statement when the superseded certificate has not been returned at the time the appeal certificate is issued:

"The superseded certificate has not been surrendered."

4. When a file sample is used, enter the following statement on the appeal certificate:

"Results based on file sample."

B. Grain (USGSA). When an appeal inspection is requested, the service is provided by an FGIS field office using the official file sample unless a new sample is requested and permitted by the regulations. All appeal results are based on a quantitative testing method. Applicants may request an appeal based on the TLC method, however, FGIS may have to forward the sample to a TLC laboratory.

In addition to the statements required in section 15.3, A:

1. Indicate "Appeal" on the certificate.

2. When a new sample is used, enter the following statement:

"Results based on new sample."

3. When reporting more than one official result on the same certificate but at different levels of inspection, explain this condition using the following statement:

"(Grade, factor, or official criteria) results based on the appeal inspection. All other results are those of the (original inspection/reinspection) service."

C. Commodities (AMA). When an appeal inspection is requested, the service is provided by an FGIS field office or CTL using the official file sample. All appeal results are based on a quantitative testing method. Applicants may request an appeal based on the TLC method, however, FGIS may have to forward the sample to a TLC laboratory.

In addition to the statements required in section 15.3, A, type the word "Appeal" in the Level of Inspection block on the FGIS-993 form or in the upper center of the original and each copy on the FGIS-957 form.

15.4 BOARD APPEAL CERTIFICATES

Upon an applicant's request, the Board of Appeals and Review performs Board appeal services for grain using a quantitative test method. Board appeal inspections are limited to the official file sample. Applicants may request an appeal based on the TLC method.

Board appeal results are reported on the same kind of certificate issued for previous testing services.

Indicate "Board appeal" on the certificate.

The Board appeal certificate supersedes other certificates issued for the lot. Enter the following statement on the Board appeal certificate:

"This certificate supersedes certificate No. (number) dated (date)."

The superseded certificate is null and void as of the date of the reinspection/retest certificate. Enter the following statement when the superseded certificate has not been returned at the time the Board appeal certificate is issued:

"The superseded certificate has not been surrendered."

Enter the following statement on the certificate when the file sample is used:

"Results based on file sample."

When reporting more than one official result on the same certificate but at different levels of inspection, explain this condition using the following statement:

"(Grade, factor, or official criteria) results based on the Board appeal inspection. All other results are those of the (original inspection/reinspection/appeal inspection) service."

15.5 APPROVED AFLATOXIN STATEMENTS

A. General. Show the following statement when certificating TLC results for rice, soybeans, and grains other than corn along with the applicable result statement from section 15.5, B.

Soybeans.

"Results based on AOAC Method 972.27, Official Methods of Analysis, 15th Edition."

Rice and Grains Other Than Corn.

"Results based on AOAC Method 972-26, Official Methods of Analysis, 15th Edition."

B. Result Statements:

1. Qualitative Service. For qualitative service, certify results as being equal to or less than a threshold (e.g., 20 ppb) or as exceeding the threshold.

"Aflatoxin exceeds 20 ppb."

"Aflatoxin equal to or less than 20 ppb."

2. Modified Qualitative Service.

"Aflatoxin exceeds (record specified level) ppb."

"Aflatoxin equal to or less than (record specified level) ppb."

"Aflatoxin exceeds 20 ppb but does not exceed 100 ppb."

"Aflatoxin exceeds 100 ppb but does not exceed 200 ppb."

"Aflatoxin exceeds 200 ppb but does not exceed 300 ppb."

"Aflatoxin exceeds 300 ppb."

3. Quantitative Service. Certify only results equal to or greater than 5 ppb to the nearest ppb. Certify results less than 5 ppb as "does not exceed" and, when test results indicate no presence of aflatoxin, certify results as "not detected."

a. Use this statement when results are less than 5 ppb.

"Aflatoxin does not exceed 5 ppb."

b. Use this statement when results are equal or greater than 5 ppb.

"Aflatoxin (record actual result) ppb."

c. Use this statement when results indicate no aflatoxin.

"Aflatoxin not detected."

d. Use this statement when Aflatest results exceed 300 ppb and the applicant does not request quantitative results certificated above that level.

"Aflatoxin exceeds 300 ppb."

C. Other Statements.

1. When certificating multiple aflatoxin results on the same certificate and the results are based on different sample types, the certificate must reflect the difference. As a guideline, the multiple results are shown as follows:

"Sublot sample results: Aflatoxin equal to or less than 20 ppb."

"Composite sample result: Aflatoxin 14 ppb."

2. Negative result statement: Upon the request of the applicant, one of the following statements may precede the applicable results statement in section 15.5, B, when the test results are equal to or less than 20 ppb.

"The aflatoxin result is negative."

"Negative Aflatoxin."

3. Use this statement when the applicant requests that the type of test be shown on the certificate:

"Results based on (indicate type of test used) method."

4. Upon request of the applicant, convert and certificate the ppb result to parts per million (ppm) using an approved statement. To convert ppb to ppm, divide the ppb result by 1,000.

"(Actual ppb result) ppb is equivalent to (converted ppm results) ppm."

5. Upon request of the applicant, convert and certificate results in micrograms per kilogram (ug/Kg) or milligrams per kilogram (mg/Kg). Use the following equivalents to determine ug/Kg or mg/Kg:

ppb = ug/Kg

ppm = mg/Kg

15.6 NOTIFYING THE FOOD AND DRUG ADMINISTRATION

The Food and Drug Administration (FDA) establishes action levels for grain, rice, pulses, and food products in the United States under the Food, Drug, and Cosmetics Act. In accordance with a cooperative agreement with that Agency, FGIS reports actionable lots to FDA.

FDA has established the action level for aflatoxin as greater than 20 ppb.

In accordance with FGIS Program Directive 906.2, Implementation of the FGIS-FDA Memorandum of Understanding (MOU), FGIS reports results that are greater than 20 ppb to FDA on official lot inspections including all export lots. Submitted sample inspections are not reported to FDA.

Report results by telephone and written confirmation to the FDA District Office as instructed in the MOU.

If a review inspection (reinspection/retest/appeal/Board appeal) is requested, do not notify FDA unless the review inspection result exceeds the 20 ppb level.

Attachment 1- "Commodity Inspection Certificate", FGIS-993

Attachment 2- "Commodity Certificate
Submitted Sample Inspection Service", FGIS-957

Attachment 1
AFLATOXIN HANDBOOK
Chapter 15
2/21/92

FGIS-993, "COMMODITY INSPECTION CERTIFICATE"

U S DEPARTMENT OF AGRICULTURE
FEDERAL GRAIN INSPECTION SERVICE

ORIGINAL
NOT NEGOTIABLE

COMMODITY INSPECTION CERTIFICATE

A-19015

DATE OF ISSUANCE (1)	ISSUED AT (2)	LEVEL OF INSPECTION (3)
APPLICANT (4)	LOCATION OF COMMODITY (5)	
IDENTIFICATION (6)	QUANTITY AND CONTAINER (7)	

(8)

I CERTIFY THAT THE SERVICES SPECIFIED ABOVE WERE PERFORMED WITH THE RESULTS STATED.	INSPECTOR (9)

This certificate is issued under the authority of the Agricultural Marketing Act of 1946, as amended (7 U S C 1621 et seq), and the regulations thereunder (7 CFR 68 1 et seq), and is receivable in all courts of the United States as prima facie evidence of the truth of the statements therein contained This certificate does not excuse failure to comply with the provisions of the Federal Food, Drug, and Cosmetic Act, or other Federal laws

WARNING: Sec 203(h) of the Agricultural Marketing Act of 1946 provides that anyone who shall knowingly falsely make, issue, alter, forge, or counterfeit any official certificate, or aid, assist, or be a party to such actions, is subject to a fine of not more than $1,000 or imprisonment for not more than 1 year, or both

The conduct of all services and the licensing of inspection/grading/sampling personnel under the regulations governing such services shall be accomplished without discrimination as to race, color, religion, sex, or national origin

FORM FGIS-993 (10-89)

Instructions for Completing Form FGIS-993, COMMODITY INSPECTION CERTIFICATE

(1) Enter the date (month, day, and year) the test was completed.

(2) Enter the name of the city and State of the field office or cooperator's office issuing the certificate (Ex: Peoria, IL).

(3) Enter the type of inspection performed (Original, Retest, Appeal).

(4) Enter applicant's name, and city and state.

(5) Enter the location of the commodity. If same as applicant in block 4 may show "Same".

(6) Enter available identification, such as carrier identification, vessel name, code, etc.

(7) a. For domestic shipments or export shipments of land carriers, enter Trucklot, Carlot, Trailer Lot, or Unit Train as applicable.

b. For export shipments enter, total weight as shown on the shiploading log.

(8) Enter the result and, when applicable, the method of testing using the appropriate statement from section 15.5 of this chapter. This section may also be used for the following information: contract, purchase order, or purchase authorization numbers and letters of credit identification. IMMEDIATELY FOLLOWING THE LAST LINE OF INFORMATION, INSERT THE FOLLOWING: "END OF RESULTS".

(9) Enter the name or signature or both of the person who issued the certificate and, if affixed by an authorized agent, the word "By" and the agent's initials.

Attachment 2
AFLATOXIN HANDBOOK
Chapter 15
2/21/92

FGIS-994, COMMODITY CERTIFICATE SUBMITTED SAMPLE INSPECTION

U S DEPARTMENT OF AGRICULTURE
FEDERAL GRAIN INSPECTION SERVICE

ORIGINAL
NOT NEGOTIABLE

COMMODITY CERTIFICATE SUBMITTED SAMPLE INSPECTION

A- 19015

DATE OF ISSUANCE (1)	ISSUED AT (2)	LEVEL OF INSPECTION (3)
COMMODITY (4)	QUANTITY IN SAMPLE (5)	
IDENTIFICATION OF SAMPLE (6)	SAMPLE SUBMITTED BY (7)	

(8)

RESULTS OF THE ABOVE INSPECTION APPLY ONLY TO THE QUANTITY OF SAMPLE INDICATED AND NOT TO THE COMMODITY FROM WHICH THE SAMPLE MAY HAVE BEEN TAKEN.

I CERTIFY THAT THE SERVICES SPECIFIED ABOVE WERE PERFORMED WITH THE RESULTS STATED.

INSPECTOR (9)

This certificate is issued under the authority of the Agricultural Marketing Act of 1946, as amended (7 U S C 1621 et. seq.), and the regulations thereunder (7 CFR 68 1 et. seq.), and is receivable in all courts of the United States as prima facie evidence of the truth of the statements therein contained. This certificate does not excuse failure to comply with the provisions of the Federal Food, Drug, and Cosmetic Act, or other Federal laws.
WARNING: Sec. 203(h) of the Agricultural Marketing Act of 1946 provides that anyone who shall knowingly falsely make, issue, alter, forge, or counterfeit any official certificate, or aid, assist, or be a party to such actions, is subject to a fine of not more than $1,000 or imprisonment for not more than 1 year, or both.
The conduct of all services and the licensing of inspecting/grading/sampling personnel under the regulations governing such services shall be accomplished without discrimination as to race, color, religion, sex, national origin, age, or handicap.

FORM FGIS-994 (5-90)

Instructions for Completing Form FGIS-994, COMMODITY CERTIFICATE SUBMITTED SAMPLE INSPECTION

(1) Enter the date the test was completed.

(2) Enter the name of the city and State of the field office or cooperator's office issuing the certificate (Ex: Baltimore, MD)

(3) Enter the appropriate level of inspection (original, retest, or appeal),

(4) Enter the type of commodity or grain tested.

(5) Enter the amount of sample submitted for testing. (Example: 1 quart, 10 pounds, 1,000 grams, etc.)

(6) Enter the sample identification assigned by the applicant, field office, or cooperator. DO NOT USE DESCRIPTIVE TERMS SUCH AS CARRIER NAME, RAILCAR PREFIXES, WAREHOUSE, LOT QUANTITY, ETC.

(7) Enter the applicant's names and complete address.

(8) Enter the result and, when applicable, the method of testing using the appropriate statement from section 15.5 of this chapter. IMMEDIATELY FOLLOWING THE LAST LINE OF INFORMATION, INSERT THE FOLLOWING: "END OF RESULTS".

(9) Enter the name or signature or both of the person who issued the certificate and, if affixed by an authorized agent, the word "By" and the agent's initials.

U.S. DEPARTMENT OF AGRICULTURE
Federal Grain Inspection Service
P.O. Box 96454
Washington, D.C. 20090-6454

CHAPTER 16

MONITORING

16.1 GENERAL

The Quality Control and Testing Branch (QCTB), Quality Assurance and Research Division (QARD), monitors all aflatoxin results determined by methods other than thin-layer chromatography (TLC). The Commodity Testing Laboratory (CTL), Beltsville Maryland, monitors TLC laboratories.

16.2 FIELD MONITORING BY QUALITY CONTROL BRANCH

Specified service points that provide aflatoxin testing service and laboratories providing aflatoxin testing services for FGIS must submit samples to QCTB for monitoring purposes.

A. Selecting Samples. Ground corn samples that have excessive moisture should not be used for monitoring purposes. (It is not necessary to determine moisture solely for the selection of monitoring samples). As a guideline, do not select samples with whole grain moisture greater than 15.5 percent. **Each week, randomly select two samples tested by quantitative method (e.g. Aflatest, etc.) and two samples tested by qualitative method (e.g. Agri-Screen, Afla-20 cup, etc.).** To request verification of a specific sample (that was not randomly selected), submit the sample as a SPECIAL CHECK sample in addition to the regular monitoring samples for the week. If there are no tests performed during the week, SEND NO SAMPLES.

B. Preparing Samples. Prepare each monitoring sample selected using the following procedures.

1. Mix the ground file sample thoroughly. Cut out a 150 gram portion.

2. Place the 150-gram portion in a paper bag or envelope and close securely. Place that bag along with the identification of the sample, test results, location of the aflatoxin laboratory and date of test inside a polyethylene bag and heat seal the bag. If a heat sealer is not available, use an alternate method to secure the polyethylene bag.

NOTE: For shipping and mailing monitoring samples to QCTB, package file samples in paper containers then secure in a plastic bag to prevent leakage during shipment. Use the plastic bags only for shipping and not for storage of the samples at field locations.

3. Send the 150-gram monitoring sample to QCTB using "Priority" or "First class mail".

4. Retain the balance of the file sample for the required retention period for review inspection purposes and for additional monitoring testing if requested by QCTB.

C. Results. If the difference between the QCTB results and the field location are statistically significant, both QCTB and the field location will repeat the test to determine whether corrective measures are required. QCTB will work with field locations to resolve any discrepancies or problems.

16.3 FIELD MONITORING BY COMMODITY TESTING LABORATORY

CTL monitors TLC results performed at field laboratories.

A. Selecting Samples. As a guideline, do not select samples with whole grain moisture greater than 15.5%. Each week select at random two samples tested by the TLC method. If there are no tests performed during the week, SEND NO SAMPLES.

B. Preparing Samples. Prepare each monitoring sample selected using the following procedures.

1. Mix the ground file sample thoroughly. Cut out a 150 gram portion.

2. Place the 150-gram portion in a paper bag or envelope and close securely. Place that bag along with the identification of the sample, test results, location of the aflatoxin laboratory, and date of test inside a polyethylene bag and heat seal the bag. If a heat sealer is not available, use an alternate method to secure the polyethylene bag.

NOTE: For shipping and mailing monitoring samples to CTL, package file samples in paper containers then secure in a plastic bag to prevent leakage during shipment. Use the plastic bags only for shipping and not for storage of the samples at field locations.

3. Send the 150-gram monitoring sample to CTL using "Priority" or "First class mail".

4. Retain the balance of the file sample for the required retention period for review inspection purposes and for additional monitoring testing if requested by CTL.

C. Results. If the difference between the CTL results and the field location results is statistically significant, CTL will notify the field location and work with them to resolve any discrepancies or problems.

U.S. DEPARTMENT OF AGRICULTURE
Federal Grain Inspection Service
P.O. Box 96454
Washington, D.C. 20090-6454

CHAPTER 17

CITE PROBE PROCEDURES

Section Number	Section	Page Number

[RESERVED]

☆U.S. Government Printing Office : 1992 - 311-370/60154

U.S. DEPARTMENT OF AGRICULTURE
Grain Inspection, Packers and Stockyards Administration
Federal Grain Inspection Service
P.O. Box 96454
Washington, D.C. 20090-6454

CHAPTER 18

VERATOX AST PROCEDURES

18.1 VERATOX AST TEST KIT

A. General. The Veratox AST test is a quick diagnostic tool to predict the presence of aflatoxin in corn and other commodities. Veratox AST measures total aflatoxins (B_1, B_2, G_1, G_2) in corn, corn germ meal, corn gluten meal, corn meal, corn soya blend, milled rice, popcorn, sorghum, soybeans, and wheat. The kit uses an enzyme-linked immunosorbent assay (ELISA) technique to obtain quantitative results from absorbance readings at 650 nm when sample readings are compared to a 20 ppb control and a pre-generated standard curve (0 to 400 ppb).

The kit is packaged in a sealed "foil bag" with a label indicating the lot number and expiration date. Record the lot number from the "foil bag" that was used for each test on the worksheet or, when applicable, designate a space on the shiploading log and record the lot number along with results.

Maintain an inventory system at each location to ensure that an adequate supply of test kits is on hand in order to provide requested service. To avoid needless disposal of unused, expired kits, do not overstock the supply at any one location.

B. Kit Contents. The following items are packaged in sealed foil and are the components that make up the test kit.

1.	Foil Pouch with:	
	Aflatoxin Antibody-coated well strips	4
	Red Marked Mixing Well Strips	4
2.	Yellow Labeled Bottle: 2 mL of 20 ppb Aflatoxin control	1
3.	Blue Labeled Bottle: 7 mL Aflatoxin HRP Conjugate	1
4.	Green Labeled Bottle: 32 mL Enzyme Substrate	1
5.	Red Labeled Bottle: 32 mL Red Stopping Solution	1

6. Reagent Boat 1

7. Instructions for Use 1

C. Precautions.

Store test kits between 36-46°F (2-8°C) when not in use. Avoid prolonged storage of kits at room temperature. Do not freeze test kits. Bring kits up to room temperature 64-86°F (18-30°C) prior to use. Do not use kit components beyond their expiration date. Do not use reagents or microwells from one kit serial number with reagents/wells from a different serial number. Reagent boats may be rinsed and reused. Only one reagent boat comes with each kit, but reagent boats can be ordered separately from the kit supplier. Do not run more than 12 wells at one time (four samples for each test). Treat all used liquids, including the sample extract, and labware as if contaminated with aflatoxins. Gloves and protective labwear must be worn at all times during the use of the kits.

18.2 PRE-EXTRACTION PREPARATION OF CHEMICALS

This test requires aflatoxin extraction from the sample with a 70 percent methanol solution.

A. Chemicals Required. Methanol and deionized or distilled water (70 percent methanol and 30 percent water).

B. Preparation.

1. Using a graduated cylinder, measure 2100 mL of methanol and place it into a clean carboy with spigot.

2. Add 900 mL deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

3. Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

4. Store this solution at room temperature in a tightly closed container until needed.

NOTE: You may prepare smaller amounts of solution if you expect slight workload as follows:

1400 mL methanol
600 mL deionized or distilled water

OR

700 mL methanol
300 mL deionized or distilled water

18.3 SAMPLE EXTRACTION PROCEDURES

A. Place a sheet of filter paper (Whatman 2V folded or S&S 24 cm pleated or equivalent) into a clean funnel mounted over a 25 X 200 mm (diameter x length) test tube or a collection beaker.

B. Label the collection container with the sample identification.

C. Place the 50-gram portion of the ground sample into the blender container.

D. Pour in 250 mL of the 70/30 percent methanol/water solution and securely close the blender top.

E. Blend for exactly two minutes at high speed.

F. Pour the resultant mixture from the blender into the funnel containing the filter paper and collect approximately 25 mL of extract.

G. Filter approximately 50 mL of water through the filter containing the ground material and allow to drain. Discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of the filtered wash in the solvent waste disposal container.

18.4 ANALYSIS

A. Test Procedures.

1. Place 3 mL of substrate (light green labeled bottle) solution into a clean, labeled reagent boat. Cover boat to protect solution from dust and light.

NOTE: Do not return any substrate solution to the original bottle once it has been removed.

2. Place 3 mL of Red Stop (red labeled bottle) solution into a clean reagent boat. Cover boat to protect solution from dust and light.

3. Open foil bag and remove 3 red-marked mixing wells for each sample to be tested (maximum of 4 samples or 12 wells). Place them in the microwell holder, and mark left end of each strip with a "1." Reseal bag by folding over and tightly closing with a suitable fastener (large paper clip, tape, or suitable dust and light protectant).

4. Place 100 mcL of conjugate (blue labeled bottle) into each mixing well using a 100 mcL pipettor with a new tip. Prime the pipette tip first before dispensing the 100 mcL. Discard the pipette tip.

NOTE: "Prime the pipette tip" is accomplished by drawing liquid up into the tip and dispensing it back into the bottle once or twice.

5. Place 100 mcL of control (yellow labeled bottle) into the first mixing well which you labeled "1." Prime the tip before dispensing. If testing more than one sample, also place 100 mcL of control into mixing well #4 for the second sample, mixing well #7 for the third sample, and mixing well #10 for the fourth sample. Discard the pipette tip.

6. Place 100 mcL of sample each in mixing wells #2 and #3. Prime the tip first before dispensing. Discard the tip. Subsequent samples should be placed in wells #5 and #6, then #8 and #9, and then #11 and #12.

See the diagram below for an example of the procedure described in steps #5 and #6.

	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
mixing wells:	O	O	O	O	O	O	O	O	O	O	O	O
	C	S1	S1	C	S2	S2	C	S3	S3	C	S4	S4

where:
"W" = well number e.g., #1 through #12
"C" = control
"S1, S2, S3, & S4" = sample numbers

7. Using the 12 channel pipettor and the overfill method (see note below), mix the contents of the mixing wells by pipetting up and down in the tips 5 times. Next transfer 100 mcL to the antibody coated wells (the unmarked, clear wells).

NOTE: The "overfill method" is performed by drawing greater than 100 mcL into the pipette tips by pressing the pipettor to the SECOND stop BEFORE placing tips into the solution. Place tips into the liquid and release the plunger slowly and completely. To dispense only 100 mcL, press plunger to the FIRST stop.

8. Mix in the antibody coated wells by gently sliding the microwell holder back and forth on a horizontal surface for 15 seconds. Be careful not to allow solution to splash out of wells. Immediately following mixing, incubate for 5 minutes. Discard all mixing (red marked) wells and tips.

9. With a wash bottle containing deionized/distilled water, fill each antibody well and dump the contents into a waste receptacle. REPEAT THIS STEP FIVE TIMES.

10. Turn microwell holder, with wells in it, upside down on a paper towel and tap gently until water is removed from the wells.

11. Using the 12 channel pipettor and the overfill method, place 100 mcL of substrate into each well.

12. Mix gently by sliding the microwell holder back and forth for 15 seconds in the same manner as step #8. Immediately following mixing, incubate for 5 minutes. Discharge the remaining substrate in the pipette tips by plunging once or twice without drawing any additional liquid up into the tips. Save these tips for the next step.

13. Using the 12 channel pipettor and the overfill method, add 100 mcL of the red stop solution (red labeled bottle) into each well.

14. Mix gently by sliding the microwell holder back and forth for 15 seconds. Again be careful not to lose any solution from the wells. Visually check the appearance of the wells. Discard all pipette tips.

B. Reading Results with Microwell Reader.

1. Turn on the power to reader at the beginning of the test procedure to allow the electronics to stabilize. Make sure that the reader is properly attached to the computer.

2. Turn on the computer and insert the VERATOX AST software disc into the drive slot.

3. Start the VERATOX program an select option A -RUN AST.

4. Check the kit identification and the standard curve values with the Standard Curve Program Calculated Points that came with the test kit. Edit standard or kit lot numbers as necessary.

5. Press the "ENTER" key, then press the "R" key to ready the computer to receive data from the microwell reader.

6. Calibrate the microwell reader by following the instructions which appear on the LCD window of the reader.

a. Remove sample carrier and press the "ENTER" key.

b. Place the filter holder in the W2 position and press "ENTER." The instrument will calibrate on the W2 filter.

c. Move the filter holder to the W1 position and press "ENTER." The instrument will calibrate on the W1 filter.

NOTE: The Micro-well reader used in the official aflatoxin testing service is designed to do several testing functions. Each function requires specific set-up parameters. The required parameters for aflatoxin testing are: "F1 set up L S P, 12S, ABSORB, N Y N." To ensure that the Micro-well reader is properly set for aflatoxin testing, periodically check the display set-up as follows:

- Press the Display Set up. Display should read F1 setup L S P 12S ABSORB N Y N.

- If the display reads differently, contact the Neogen Corporation representative for instruction.

- Otherwise, press the Display Set up again. This will return the instrument to normal operational mode.

d. Press the "CLEAR" key, then the "BLANK" key. This will blank the instrument on air and it is now ready to measure absorbance.

e. Place the wells into the reader's sample holder. Make sure that the well marked "1" is in the far left position in the holder.

f. Move the holder to the left so that the first well is under the reader and press the "READ" key. Repeat this process until all wells are read.

g. Follow the instructions as requested by the software. Values displayed on the computer screen will be the mean of the duplicate measurements.

18.5
EQUIPMENT, SUPPLIES, AND SAFETY

A. EQUIPMENT AND SUPPLIES

Blender	Oster mixer, Model 848-31A; Oster Corp., or Waring Blender with S.S. blender container or similar.
Cutting Assembly	Process unit with sealing ring for Oster Mixer, Model 848-31A; Oster Corp. 937-45 or Eberbach blender jar or similar.
Bottom cap	Threaded for Oster Mixer, Model 848-31A; Oster Corp. No. 937-46.

Square type jar	Designed to fit above.
Nalgene funnels	80 mm Top I.D., Stem 30 mm, Stem O.D. 18 mm; American Scientific Products No. F7465-2
Culture tubes	Disposable - 25 mm x 200 mm; with Teflon Lined Caps; American Scientific Products No. 14-915H
Test tube rack	Capable of holding 25 mm x 200 mm culture tubes; 11-1/2" x 4-3/4" x 3-5/8"; American Scientific Products No. S9224-4
Filter paper	24 cm diameter; American Scientific Products No. F-2875-24
Cylinders	Polypropylene, graduated 1000 mL capacity\ 250 mL capacity
Carboy	Nalgene, polyethylene, with spigot, 2 gallon capacity; Fisher Scientific No. 02-936-6A
Vortex Mixer	American Scientific Products No. S822S

(Optional)

Timer (5-minute capacity)

Refrigerator (2-8°C)

Markers: Sharpie or equal (permanent ink that will not wash off)

Absorbent material: Kim wipes or paper towels

Waste Receptacle: Plastic 1/2 gallon bucket

Wash Bottle: 250 mL plastic squeeze bottle

Microwell Strip Reader: BioTek EL301 or equivalent

IBM Compatible Computer

Multichannel Pipettor: TiterTek 12 channel or equal

Pipettor and Pipette Tips (100 mcL): Pipetteman, MLA or equivalent

Pipettor and Pipette Tips (1 mL): Pipetteman, MLA or equivalent

Microwell Holder

CHEMICALS

Methanol

Deionized or distilled water

B. SAFETY ITEMS

Disposable impermeable gloves

Disposable fire retardant laboratory coats

Chemical splash goggles

Storage cabinet, flammable materials

Fire extinguisher, dry powder

Fume hood

Eyewash fountain